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Investigating the Impact of Smoking in the Post-COVID-19 Era on Young People: DNA Damage Analysis Using the Comet Assay

Elaheh Abiri[1,2], **Rasoul Raesi**[2,3*]

[1.] Department of Cellular and Molecular Biology, School of Biology and Institute of Biological Sciences, Damghan University, Damghan, Iran.
[2.] Department of Public Health, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran.
[3.] Department of Public Health, School of Health, Torbat Jam Faculty of Medical Sciences, Torbat Jam, Iran. Email: Raesi.br881@iau.ac.ir

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Abstract

Background and Aim: The COVID-19 pandemic not only challenged immune and respiratory systems but also reshaped health-related behaviors, including tobacco use. Increasing evidence suggests that cigarette smoking exacerbates oxidative stress and genomic instability in COVID-19 patients. This study aimed to investigate the impact of smoking on DNA damage among young adults in the post-COVID-19 period using the Comet Assay technique.

Methods: This analytical study included 45 male COVID-19 patients (under 60 years old) who were active or former smokers and were hospitalized in May 2024 at a university-affiliated hospital in Sari, Iran. A comparison group consisted of 42 age-matched non-smoking COVID-19 patients, and 25 healthy non-smoking, non-infected individuals served as the control group. Peripheral blood samples were collected from all participants, and DNA damage in leukocytes was assessed by Single Cell Gel Electrophoresis (Comet Assay). Statistical analyses were performed to compare the extent of DNA damage among groups.

Results: The results showed a significant increase in DNA damage indices—including tail length and tail DNA percentage—in smoking COVID-19 patients compared with both non-smoking patients and healthy controls ($p < 0.001$). Former smokers also exhibited elevated but comparatively lower levels of DNA damage. These findings suggest that cigarette smoking, even after recovery from COVID-19, can exacerbate oxidative stress and impair DNA repair mechanisms, leading to persistent genomic instability. The Comet Assay proved to be a sensitive and valuable biomarker for detecting subtle DNA damage and monitoring post-viral cellular health.

Conclusion: Smoking remains a significant contributor to genomic injury in young adults recovering from COVID-19.

Keywords: COVID-19; Smoking; DNA Damage; Comet Assay; Post-COVID-19; Genomic Instability

1. Introduction

The global pandemic of COVID-19 has not only caused an acute infectious and respiratory crisis, but has also produced significant long-term biological and behavioral impacts across various populations. The "post-COVID-19 era" is a multidimensional concept that includes physiological, psychological, and behavioral consequences, including changes in substance use patterns (e.g., smoking), increased oxidative stress, and chronic inflammatory disorders that may persist for months or years after infection. Attention to younger groups is important: young

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people under 60 years of age, despite being less likely to experience acute mortality, may be affected by molecular and genomic consequences that increase the burden of chronic diseases in the future. Investigating the interaction between smoking and post-SARS-CoV-2 molecular consequences, including DNA damage, is essential for designing preventive interventions and optimizing public health policies [1-5]. At the molecular level, two main mechanisms explain the increased genetic damage in patients with COVID-19: oxidative stress and systemic inflammation. SARS-CoV-2 infection can cause excessive production of reactive oxygen species (ROS) and reactive nitrogen species; these molecules directly lead to the formation of DNA lesions by inducing oxidation of sugars, lipids, and nucleic acids. Clinical and laboratory studies have reported increased oxidative parameters and markers of DNA damage in COVID-19 patients. At the same time, cigarettes are known to be a rich source of oxidants, free radicals, and numerous genotoxic chemicals (such as benzopyrene) that can directly induce DNA breaks and mutations, and additionally disrupt DNA repair processes. The intersection of these two factors—systemic viral infection and exposure to cigarette smoke—can produce spikes and cumulative effects in gene damage that have long-term consequences for an individual's genomic health [5-9]. Several reasons led us to focus on a younger population (<60 years of age): [1] younger people in many societies have high rates of smoking or are more likely to initiate or increase smoking in response to the socio-economic pressures of the pandemic; [2] early onset of genetic damage may provide a longer window for chronic outcomes (chronic inflammation, metabolic diseases, late-stage cancers); and [3] epidemiological data suggest that despite lower mortality, long-term molecular consequences are observed in individuals recovering from COVID-19 , which require monitoring and clarification of mechanisms. As a result, studying young people allows us to provide a sensitive window for preventive interventions and health education. Several factors are involved in the pathway between smoking and DNA damage in COVID patients: both smoking and viral infection can increase ROS production; ROS cause single- and double-stranded DNA breaks, oxidation of bases such as 8oxoG-, and creation of recognition sites for repair enzymes. Studies using biomarkers and Comet assays have reported a clear increase in DNA damage parameters in COVID patients [4, 10–14]. Evidence suggests that cigarette compounds can inhibit important components of repair pathways such as NER and BER or reduce the expression of key proteins; this reduction in repair leads to the accumulation of lesions and an increased probability of recombination errors, which predispose to genomic instability. Recent findings have also reported a decrease in expression of some repair genes in response to cigarette smoke [15-19]. The prolonged inflammatory response following viral infection can increase the production of pro-inflammatory cytokines; these cytokines, in turn, can alter cellular metabolic and antioxidant pathways and create conditions for persistent genetic damage. The association between inflammatory markers, oxidative stress, and the extent of DNA damage in patient samples has been reported [3, 20]. The comet assay is a sensitive, relatively simple, and inexpensive method for measuring DNA breaks at the single-cell level. This technique can indicate the amount and type of damage (e.g., single- and double-strand breaks, oxidative lesions in modified versions) and can be used cost-effectively in clinical epidemiological studies. In numerous studies of COVID-19, the Comet assay has been repeatedly used to detect increased tail length and % tail DNA in leukocytes of patients, making it a suitable tool for monitoring genotoxicity after infection. The capacity of the Comet assay to detect relatively small differences between groups allows for the assessment of the cumulative effects of smoking and viral infection [21-26]. Over the past three years, several clinical and laboratory studies have shown that patients with COVID-19 exhibit significantly increased indices of DNA damage and oxidative parameters. Some studies have reported increased % tail DNA in affected groups compared to controls using peripheral blood samples and the Comet assay; in others, the severity of the disease has been correlated with the degree of genetic damage. Reviews also show that cigarette smokers often experience higher disease severity and adverse outcomes, that can be linked to increased genetic damage [5, 27-29]. However, specific data that comprehensively demonstrate the impact of smoking on DNA damage in the post-COVID period in young populations are still limited and require targeted studies. Despite evidence separately examining each of the three components of smoking, COVID-19, and DNA damage, there is a significant lack of studies that evaluate these three factors synergistically in a target population (especially young people). Key questions that justify this research context include: Does smoking lead to a significant increase in genetic damage in patients recovering from COVID-19? Are there cumulative effects between smoking history and disease severity? And can the Comet assay serve as a predictive marker of long-term genomic outcomes in these patients? Answers to these questions could guide clinical strategies (e.g., molecular monitoring, targeted smoking-cessation counseling) and public health policy. If smoking after SARS-CoV-2 infection causes persistent increases in DNA damage, this would mean

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that a large population of recovered individuals—especially those who were or are current smokers—are at increased risk of chronic disease and genomic instability processes. These consequences go beyond individual concerns and have broader population health implications, long-term health costs, and a need for intervention programs, such as smoking-cessation plans, molecular monitoring, and targeted antioxidant therapies. Therefore, the results of the studies that accurately characterize the extent of DNA damage according to smoking status, clinical severity, and biochemical parameters can help identify at-risk groups and develop preventive interventions. The main innovation of the proposed study lies in three axes: 1) integrating genotoxicity assessment (Comet assay) with clinical parameters and smoking history in a young population; 2) focusing on the "post-COVID-19 period" to characterize residual effects rather than acute infection alone; and 3) using the data to provide practical clinical and policy recommendations, such as molecular monitoring and focused smoking-cessation programs for COVID-19 recoveries. This approach can bridge the gap between molecular research and health policy and show how traditional epidemiological methods, combined with molecular biomarkers, can guide targeted interventions. In summary, the combination of oxidative effects from smoking and the inflammatory/oxidative response induced by SARS-CoV-2 infection can lead to a serious genomic problem in post-COVID recovered individuals. Focusing on young populations and using a sensitive tool such as the Comet assay to measure DNA damage provides an opportunity to identify long-term genotoxic consequences and design targeted preventive and therapeutic interventions. Careful studies and longitudinal follow-ups are needed to determine the magnitude of this risk, its persistence over time, and the response to protective interventions such as smoking cessation or oxidative stress-reducing therapies.

2. Methods

Overview of Study Design

This study is an analytical/epidemiological investigation with a molecular component. Participants were divided into three groups: (1) Case group- COVID-19 patients under 60 years of age who were smokers (active or former) (n = 45); (2) Comparison group- COVID-19 patients under 60 years of age who had never smoked (n = 42), and (3) Control group- healthy individuals under 60 years of age who are non-smokers and have not been infected with COVID-19 (n = 25). Then, by collecting peripheral blood samples, the amount of DNA damage in leukocytes was measured. Blood sampling was collected 4 to 12 weeks after documented clinical recovery and a negative PCR test. Participants sampled earlier than 4 weeks or later than 12 weeks were excluded. The use of Comet Assay (alkaline version), as the main genotoxic assay tool with optimized conditions and internal controls, represents the methodological innovation of this part. Participants were enrolled between May 2023 and November 2024. They were selected from volunteers who had visited Sari Hospital, Iran, as outpatients or inpatients. Participants whose SARS-CoV-2 infection was confirmed by RT-PCR were included in the case group, while the COVID-negative group consisted of participants with no known infection and confirmed by serial negative serology. The inclusion criterion in this study was the absence of underlying diseases such as cardiovascular diseases, metabolic disorders, etc. On average, participants reported smoking 16.8 ± 6.2 cigarettes per day for 14.3 ± 5.7 years, corresponding to a mean exposure of 11.9 ± 6.4 pack-years. The distribution of smoking intensity showed that 20% of participants smoked 1–10 cigarettes per day, 59% smoked 11–20, and 21% smoked more than 20 per day. Pack-year stratification indicated that 9% had fewer than 5 pack-years, 14% had 5–10 pack-years, 38% had 10–20 pack-years, and 64% had more than 20 pack-years. Of the 45 smokers, 33 were active smokers and 12 were former smokers; among former smokers, 7 had quit within the past year, 3 had quit 1–5 years earlier, and 2 had been abstinent for more than 5 years. The study protocol was in accordance with the Declaration of Helsinki, and ethical approval was granted by the Institutional Review Boards of the Ethics Committee of Damghan Azad University (IR.IAU.DAMGHAN.REC.1400.005). Written informed consent (or proxy consent for participants with cognitive impairment) was obtained from all subjects. The design of the method followed MIRCA (Minimum Information for Reporting Comet Assay) principles to ensure reproducibility and comparability of results.

Sample preparation and participant selection

Blood sample collection: 5 to 10 ml of peripheral blood was collected from each participant and injected into a tube containing an anticoagulant called EDTA. Samples were transported to the laboratory within 1 hour and stored at 4°C. Leukocyte isolation: Using a density fractionation centrifuge (Ficoll–Paque), PBMC (Peripheral Blood Mononuclear Cells) isolation was performed. After isolation, the cells were washed twice with cold PBS buffer (pH ≈ 7.4) to remove residual plasma or platelets. The cells were counted and diluted to the appropriate density (e.g., 1 × 10^6 cells/mL).

Comet Assay Protocol

First, a base gel was prepared from 1% normal agarose in TBE buffer, melted at 90–95 °C, and cooled to

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approximately 60 °C before being poured onto pre-cleaned SuperFrost Plus slides to ensure optimal adhesion. For cell embedding, approximately 1×10^5 leukocytes were mixed with 100 µL of 0.5% low-melting-point agarose at 37–40 °C. The cell–agarose suspension was then spread as a uniform layer and allowed to solidify at 4 °C for 5 min. Slides were subsequently incubated in lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, and 10% DMSO) for at least 1 h, preferably overnight, at 4 °C in the dark to minimize oxidative damage. After lysis, gels were washed twice for 5 min each in neutralization buffer (0.4 M Tris, pH 7.5). Alkaline unwinding was performed using 300 mM NaOH and 1 mM EDTA (pH > 13) for 20–30 min at 4 °C, followed by electrophoresis in the same buffer at 1 V/cm (typically 25 V) for 20 min, while maintaining the buffer temperature below 4 °C. Slides were then neutralized in 0.4 M Tris buffer (pH 7.5) for 5–10 min and stained for 20–30 min in the dark with SYBR Green, ethidium bromide (2 µg/mL), or propidium iodide. Imaging and scoring conditions, listed as N/A for time or temperature, were performed under a fluorescence microscope (20×–40× objective) equipped with a CCD camera. Analysis was carried out usin OpenComet, scoring software, evaluating at least 50–100 nuclei per slide. Quality control included both negative (untreated) and positive (H_2O_2-treated) controls in every run to reduce inter-assay variability.

Data Analysis and Comet Scoring Criteria

The main parameters extracted from the software included Tail Length, % Tail DNA, and Olive Tail Moment (the product of tail length and % DNA). For each sample, at least 50 to 100 cells were scored to provide a reliable statistical distribution. Comparisons between groups were performed using either nonparametric or parametric tests, depending on the data distribution (Mann-Whitney test, t-test, and ANOVA). Each run included a negative control (untreated sample) and a positive control (sample subjected to controlled damage with H_2O_2). During the experiment, possible sources of variability (e.g., temperature changes, voltage difference, gel thickness variations) were documented and taken into account in the interpretation of the results. In reporting, all experimental conditions (agarose concentration, lysis time, pre-equilibration time, electrophoresis voltage and duration, number of cells, and software filters) were presented according to MIRCA recommendations to allow reproducibility.

3. Results

The comparison of DNA damage indices between the smokers and non-smokers (under 60 years old) in the post-COVID-19 era is shown in Figure 1. The results show that the mean tail length and percentage of DNA in the tail in the COVID-19 smoker group were significantly higher than those in the other groups ($p < 0.001$), indicating an increase in single-strand and double-strand DNA breaks. The increase in OTM also indicates a greater severity of genetic damage and an abnormal distribution of DNA fragments in the electric field. The standard error bars indicate the inter-sample standard deviation, and the statistical values were examined using one-way analysis of variance (Anova) and Tukey's post hoc test. As shown in Figure 1, DNA damage indices, including tail length, tail DNA percentage, and olive tail torque, were significantly higher in the COVID-19 smoker group compared with the other two groups. The mean tail length and tail DNA percentage in this group were 43.7 ± 5.58 µm and 42.2 ± 5.05%, respectively, approximately 1.5- to 2-fold higher than those in non-smokers with COVID-19. The healthy control group showed significantly less DNA damage of 14.4 ± 3.13 µm for tail length and 12.8 ± 3.73% for tail DNA, indicating normal genomic stability in uninfected non-smokers. One-way analysis of variance (ANOVA) showed that the differences between the three groups were significant for all three indices ($p < 0.001$). Tukey's post hoc test showed that all pairwise comparisons (COVID-19 smokers vs. COVID-19 non-smokers, and both vs. controls) were significantly different ($p < 0.001$). Also, a strong positive correlation was observed between tail length and percentage of DNA in the tail ($r = 0.88$, $p < 0.001$), indicating a simultaneous increase across different parameters of DNA damage. In COVID-19 patients who smoked, the severity of respiratory symptoms (chronic cough, shortness of breath, SpO_2 below 90%) was significantly higher than in the other groups. In contrast, non-smokers with COVID-19 showed minimal clinical manifestations. Control subjects had milder symptoms, a faster recovery rate, and no detectable genetic damage. The overall correlation between tail length and percentage of DNA in the tail in the entire dataset was $r = 0.85$, $p \approx 9.25\times10^{-33}$ (Pearson). This strong and significant correlation indicates a simultaneous increase in different parameters of DNA damage (Figure 2). This strong correlation suggests that both comet indices (migration rate and fraction of migrated DNA) convey integrated messages of genomic damage, and one can be used to support the other in epidemiological analyses. The distribution of tail length in the study groups (box plots) is shown in Figure 3. As revealed in Figure 4, hypoxia (decreased SpO_2) in smoking patients may be one of the mechanisms accelerating genomic damage. The Pearson correlation matrix between the DNA damage indices and clinical

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variables is presented in Figure 5.

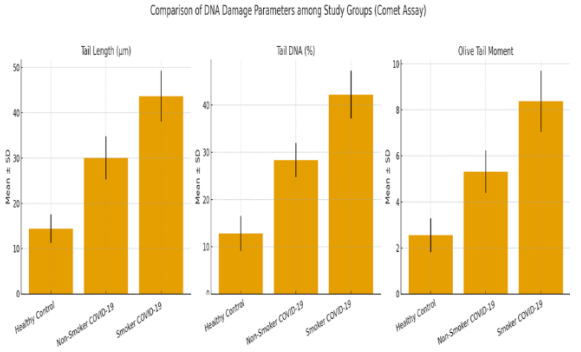


Figure 1. Comparison of DNA damage indices between smoking and non-smoking young adults (<60 years) groups in the post-COVID-19 era based on the Comet assay. Bar graphs show the mean ± standard deviation (SD) of three main DNA damage indices in peripheral blood leukocytes: (A) Tail Length (µm), (B) %Tail DNA, and (C) Olive Tail Moment (OTM). The three groups studied were: smoking young adults (<60 years) with COVID-19 (COVID-Smokers), non-smoking young adults (<60 years) with COVID-19 (COVID-Non-Smokers), and healthy non-smoking controls (Healthy Controls).

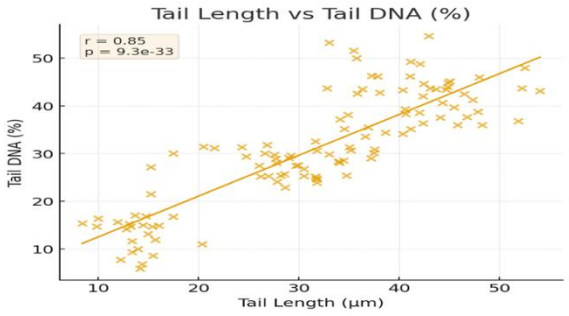


Figure 2. Scatterplot of tail length (µm) versus tail DNA (%) across all participants (n = 112). The solid line represents the linear regression. The Pearson correlation coefficient between the two variables is r = 0.85 with a p value of ≈ 9.3×10^{-33}, indicating a strong positive correlation between the different DNA damage indices. The dots represent individual observations; for better readability, α = 0.7 has been applied. This correlation indicates the synergy of genotoxicity manipulations and indicates that increases in different parameters (Comet assay) occur simultaneously.

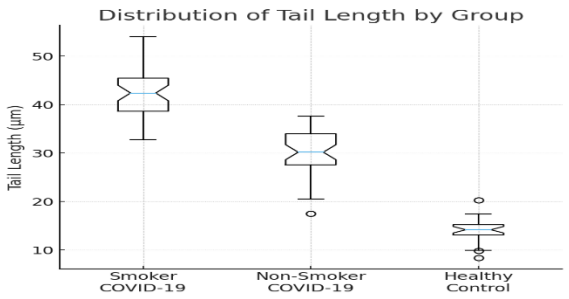


Figure 3. Boxplot of tail length by group. Distribution of tail length (µm) in three study groups: smokers with COVID-19, non-smokers with COVID-19, and healthy controls. Boxplots with notches indicate medians, quartiles, and outliers. The "smoker" group shows a higher mean and median, which are significantly different from the other two groups. This pattern is consistent with increased genotoxicity due to the combined effect of smoking and the viral inflammatory process.

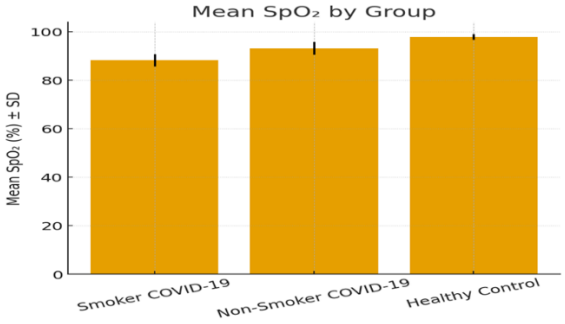


Figure 4. Graph of mean ambient oxygen saturation SpO_2 (percentage) ± standard deviation for each group. The "smoker with COVID-19" group had a lower SpO_2 value on average compared to the non-smoker and control groups; this clinical difference could lead to increased intracellular oxidative stress and subsequent DNA damage. Statistical tests showed a significant difference in means ($p < 0.01$).

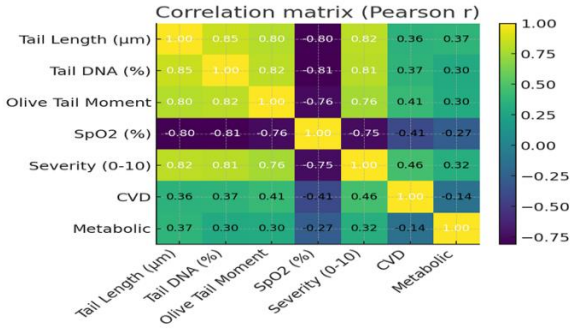


Figure 5. Correlation heatmap (matrix): Pearson correlation matrix between key study variables: tail length, percentage (DNA) in tail, (Olive Tail Moment), SpO_2, symptom severity (Severity), and comorbidity markers (CVD, (Metabolic). Numbers in boxes indicate correlation coefficient r. Note that Comet variables are highly correlated with each other (r ≈ 0.75–0.85); SpO_2 is inversely correlated with DNA damage indices (negative r), and comorbidities (especially CVD) are moderately positively correlated with Olive Tail Moment. This overall pattern suggests that the interaction between clinical status (hypoxia, symptom severity) and biological parameters (Comet indices) is logically and biologically meaningful.

4. Discussion

In this study, the findings showed that smoking in the post-COVID-19 period significantly increased DNA damage in peripheral blood leukocytes of young people. The "smoker with COVID-19" group had approximately one and a half times higher values for the three main indicators- Comet tail length, Tail DNA

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percentage, and Olive Tail Moment- compared to the non-smoker group with COVID-19. These indicators were also significantly higher compared to the healthy control group. These findings are consistent with some international studies showing that the severity of COVID-19 disease, the inflammatory state, and the oxidative stress associated with it are all linked to increased genetic damage and impaired DNA repair response [30, 31]. In addition, a positive association was found between smoking history, severity of clinical symptoms (especially hypoxia and more severe respiratory symptoms), and increased DNA damage. This association suggests a synergistic effect of smoking and viral disease on genomic health. One study reported that patients with COVID-19 had increased DNA damage compared to those not suffering from it, with more severe cases showing greater damage. Another study showed that parameters of oxidative stress and immune response measured with Comet assay were significantly increased in patients with SARS-CoV-2 compared to controls [2, 17–33]. This evidence is consistent with our results. Another study showed that despite DNA damage, DNA repair pathways (such as Base Excision Repair and Double-Strand Break Repair) were activated and their levels were increased in patients with SARS-CoV-2, which can be an adaptive response to the molecular damage caused by the infection (34, 35). These findings are consistent with our observation that markers of DNA damage were higher in smokers, but the possibility of repair pathways is also possible. Combining the results of this study with recent evidence, we suggest the following mechanisms to explain the increased DNA damage in the smoking group after COVID-19. Smoking itself is a potent source of ROS, and SARS-CoV-2 infection induces a systemic inflammatory response along with the production of cytokines such as IL-6 and TNF-α, which increase ROS production. These ROS cause single- and double-strand breaks in DNA, oxidation of nucleotide bases, and the formation of damaged bases such as 8-oxo-G. In addition, cigarette compounds can reduce the expression or activity of repair enzymes such as those involved in Base Excision Repair (BER), Nucleotide Excision Repair (NER), and the Double Strand Break Repair (DSBR) pathway. SARS-CoV-2 has also been shown to directly interfere with DNA damage response (DDR) pathways in some studies. For example, one study found that the virus had proteins that disrupted repair processes and contributed to DNA damage. Microcline formation and increased Comet tails were also reported in tissues and blood sample from human patients.[30] If smokers are exposed to hypoxia, ventilation, or other metabolic stressors during the acute phase of COVID-19, this can lead to increased endogenous ROS production and more severe DNA damage. Repeated DNA damage and incomplete repair can cause cellular senescence, which is a source of inflammatory cytokine production. Studies have shown that the DNA repair response is increased in active COVID-19 patients, but if this response is chronic and persistent, it can result in long-term inflammation and genomic instability [30-35]. A study that examined DNA methylation changes after COVID-19 showed widespread changes at CpG sites, even when smoking status was controlled in the models. Furthermore, the mechanisms proposed in SMR/TWAS studies indicated that smoking may affect the expression of genes that, from a biological perspective, increase tail length and OTM in the smoking group with COVID-19. This is likely due to the synergy of two main factors. Increased reactive oxygen species (ROS) generated by smoking and the inflammatory response caused by the virus (SARS-CoV-2) act together with reduced efficiency of DNA repair pathways in cells involved in chronic oxidative stress (28, 30). These results are consistent with recent evidence in the field of post-COVID genotoxicity and the long-term effects of smoking on genomic health. In the results of this study, the inset micrograph presented microscopic images obtained from the Comet assay in representative cells from each group, indicating that DNA migration (star tail) in cells of the COVID-Smoker group was clearly greater and more extensive compared to the healthy control group. In contrast, cells from the non-smoking group with COVID-19 showed only minor DNA breaks. These findings suggest that smoking in the post-COVID-19 period not only impairs DNA repair but may also perpetuate genomic damage, which in the long term increases the risk of chronic diseases associated with genotoxicity. These findings suggest that smoking can cause persistent genomic damage in the blood cells of COVID-19 patients by inhibiting DNA repair pathways. In fact, the combination of viral infection with exposure to toxic compounds in cigarettes has a strong synergistic effect on genetic damage. The significant increase in the Olive Tail Moment index in smoking patients indicates the spread of single-stranded and double-stranded DNA damage. From a pathophysiological perspective, this could lead to activation of chronic inflammatory pathways, increased apoptosis, and even increased susceptibility to respiratory cancers in the post-Covid period. DNA damage, if not repaired or incompletely repaired, can result in mutations. Acceleration of cellular senescence, which can lead to reduced tissue repair capacity, increased chronic inflammatory damage and contributed to dysfunction of organs including the lungs, heart, and blood vessels. This sets the stage for multiple post-COVID complications including chronic fatigue, reduced respiratory capacity, reduced

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cardiovascular function, and metabolic disorders. Those who have been smokers are more likely to experience these consequences. This indicates the importance of smoking cessation, antioxidant interventions, or drugs which improve DNA repair responses, especially in patients recovering from COVID-19. Focusing on people under 60 years of age allows early effects to be recognized before chronic consequences become prominent. Having non-smokers with COVID and healthy controls allows us to separate the effects of the virus from those of smoking. The use of indicators such as %Tail DNA, Olive Tail Moment, and Tail Length has provided greater precision in the data analysis. Adjusting for overlapping variables, such as age, inflammatory status, and clinical stressors, in the analyses has reduced confounding errors. Although the study is carefully conducted, there are limitations that must be considered. Despite the fact that sample sizes of 45 smokers, 42 non-smokers, and 25 healthy controls seem appropriate, statistical power may not be sufficient for some regression analyses or examining multiple interactions, especially for subgroups with more severe disease. Moreover, a certain amount of time is needed between recovery from COVID-19 and sample collection for the post-infection effects to stabilize. Lack of precise control over recovery time may have caused overlap between acute injury and long-term effects. Furthermore, although the alkaline version is highly sensitive for detecting single-strand breaks, alkali-labile sites, and incomplete excision repair sites, it does not distinguish among specific classes of DNA lesions. In particular, oxidative base modifications such as 8-oxoguanine cannot be selectively quantified without incorporating lesion-specific enzymes such as formamidopyrimidine DNA glycosylase (FPG) or endonuclease III. Consequently, while the elevated Comet parameters observed in the smoker post-COVID group indicated increased DNA strand breakage, the assay cannot specify the contribution of oxidative versus non-oxidative mechanisms, nor can it identify mutation-relevant base alterations. Future studies incorporating enzyme-modified Comet assays or complementary biomarkers of oxidative stress (e.g., 8-oxoG quantification, γ-H2AX, or LC–MS/MS–based lesion profiling) can provide a more detailed characterization of the underlying types of DNA damage.

5. Conclusion

In conclusion, the results of this study suggest that smoking significantly increases genomic damage in post-COVID-19 young adults (<60 years). The combination effects of smoking and viral/infl - amatory stress suggests the possibility of persistent DNA damage and an increased risk of chronic disease. These findings emphasize the need to address molecular consequences after infection. It is further recommended that smoking cessation programs, post-recovery molecular care, and continued research in these areas be implemented. The findings may also inform public health policies by identify smokers recovering from COVID-19 as a high-risk group and providing them with specific support resources.

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Ethical Considerations and Compliance with Ethical Guidelines

This study was approved by the Ethics Committee of Damghan University (IR.DU.REC.1403.016). Since anonymized bacterial isolates were used, no patient identifiers were collected, and informed consent was waived. No animal procedures were performed in this study.

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Conflict of interest

The Authors declare that there is no conflict of interest.

AI Using Declaration

During the preparation of this work, the authors used ChatGPT in order to check the grammar and improve readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Author's contributions

The authors equally contributed to preparing this article.

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