

EFFECTS OF VITAMIN C SUPPLEMENTATION ON OXIDATIVE DNA DAMAGE AND OXIDATIVE STRESS MARKERS IN NARGHILE SMOKERS AND NON-SMOKERS

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ABSTRACT

Narghile (Hookah) smoking is increasingly popular among young adults and is associated with exposure to toxicants that contribute to oxidative stress, DNA damage, and Cellular dysfunction. This study aimed to investigate the effects of Narghile smoking on Oxidative stress and DNA damage levels in Narghile smokers compared with nonsmokers. It provides valuable insights into the antioxidant and/or prooxidant effects of Ascorbic acid. The study objective was to evaluate: 8-hydroxy-2'-deoxyguanosine, malondialdehyde, Vitamin C concentration, and Total antioxidant capacity, which were measured before and after one month of daily vitamin C supplementation (500 mg/day) in Narghile. The Biomarkers were measured using a standardized spectrophotometric microplate reader, an ELISA technique, and a manual Thiobarbituric acid assay for Malondialdehyde assessment. Ninety-seven healthy adult males and females were randomly recruited from Duhok city. This study used a cross-sectional design for comparison and a quasi-experimental component to examine the effect of vitamin C in Narghile smokers. Statistical analysis included independent t-tests. Serum biomarker levels of 8-hydroxy-2'-deoxyguanosine and malondialdehyde were significantly higher in Narghile smokers than in non-smokers ($p < 0.0001$). After vitamin C supplementation, malondialdehyde levels significantly decreased among smokers ($p < 0.0001$), whereas 8-hydroxy-2'-deoxyguanosine levels markedly increased ($p < 0.0001$), indicating enhanced oxidative DNA damage. Total antioxidant capacity increased slightly, and serum vitamin C concentrations remained lower in smokers than in non-smokers throughout the study. Findings suggest that although vitamin C reduces lipid peroxidation, it paradoxically increases oxidative DNA damage in smokers, potentially due to a pro-oxidant effect mediated by smoke-related metal ions.

KEYWORDS: 8-hydroxy-2'-deoxyguanosine, Genomic instability, Lipid peroxidation, Narghile smokers, Total antioxidant capacity.

1. INTRODUCTION

Narghile smoking, also known as Waterpipe or Hookah smoking, is a traditional way of smoking tobacco that has become increasingly common in popularity worldwide over the past few decades, particularly among adolescents and young adults. Once confined to specific regions, it has now become a global trend and is frequently perceived as a safer alternative to cigarette smoking. However, mounting evidence suggests that this perception is misguided. Narghile smoke delivers substantial amounts of nicotine, tar, carbon monoxide, and other harmful toxicants that pose serious health risks.¹

Globally, tobacco use remains the second leading cause of death, accounting for approximately five million fatalities annually, roughly one in every ten adult deaths.² Despite this alarming statistic, Narghile smoking continues to be socially accepted due to misconceptions.³ These misconceptions contribute to its increasing popularity, especially in cultures where it is a traditional or communal practice.

Emerging evidence has drawn attention to the biological consequences of Narghile smoking, particularly its potential to induce oxidative stress. Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, leading to cellular damage, including to DNA. One well-established biomarker for oxidative DNA damage is 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is not only mutagenic but also implicated in carcinogenesis.⁴ Narghile smoke has been found to generate ROS and other toxicants at levels comparable to or even exceeding those produced by cigarettes, thereby promoting oxidative lesions in DNA.⁵

Several studies have demonstrated that narghile smoking significantly reduces total antioxidant capacity (TAOC), weakens endogenous antioxidant defenses, and elevates oxidative stress markers, including malondialdehyde (MDA), a key indicator of lipid peroxidation.⁶ One study conducted among healthy Saudi males revealed that Narghile smokers exhibited reduced serum levels of vitamin C and TAOC, suggesting increased oxidative burden and a potentially heightened risk for cardiovascular complications.⁷

Antioxidants play a crucial role in neutralizing ROS and maintaining redox homeostasis. Among these, ascorbic acid (vitamin C) is a prominent antioxidant known for its dual role; it can act as both an antioxidant and a pro-oxidant depending on its concentration and the surrounding biochemical environment.⁸ At higher concentrations,

vitamin C may reduce metal ions such as iron or copper, which can then react with hydrogen peroxide to produce hydroxyl radicals via Fenton reactions, exacerbating oxidative stress.⁹ Consequently, smoking may promote oxidative damage not only through ROS inhalation but also by depleting antioxidant reserves, thereby rendering lipids, proteins, and DNA more vulnerable to oxidative injury.¹⁰

Despite the well-documented toxicological effects of cigarette smoke, research specifically exploring the genotoxic impact of Narghile smoking remains limited. This scarcity of evidence fosters a false sense of safety in communities where Narghile is commonly used. Whereas some studies have linked Narghile smoking to oxidative DNA damage, they are often constrained by small sample sizes, weak study designs, or insufficient biomarker profiling.¹¹ Notably, no studies have directly investigated the modulatory role of vitamin C, either as an antioxidant or pro-oxidant, in Narghile smokers compared to non-smokers.

Given the increasing prevalence of waterpipe use in this region and the lack of robust local data on its molecular effects, this study aims to fill an important knowledge gap. Specifically, it seeks to evaluate the potential genomic instability associated with Narghile smoking by measuring serum levels of 8-OHdG, TAOC, MDA, and vitamin C. By comparing these oxidative stress markers in Narghile smokers and non-smokers, both before and after vitamin C supplementation, this study will contribute critical insights into the complex role of vitamin C in modulating oxidative stress in the context of Narghile tobacco exposure.

2. MATERIALS AND METHODS

2.1 Study Design and Ethical Considerations

The study was conducted using an interventional cross-sectional design that included Narghile smokers and nonsmokers. In addition, a quasi-experimental approach was applied to evaluate the effect of one month of vitamin C supplementation among Narghile smokers and systematically compare multiple oxidative stress and antioxidant biomarkers among two groups of both genders aged 18–30 years:

Group 1: 50 apparently healthy regular Narghile smokers.

Group 2: 47 apparently healthy non-smokers.

Narghile smokers were evaluated before and after a one-month intervention with Pure vitamin C supplementation (500 mg/day).

Ethical approval for the study was obtained from the General Directorate of Health in Duhok Governorate, and informed consent was secured from all participants prior to enrollment. The study also received approval from ClinicalTrials.gov (ClinicalTrials.gov Identifier) before any data collection. All required transformations to the study protocol were submitted to the Research Ethics Committee for re-approval, thereby assuring the avoidance of discrepancies and maintaining transparency in the conduct of the research. Individuals with a history of chronic diseases (e.g., diabetes mellitus, thyroid disorders, malignancies) or who had recently consumed any dietary supplements were excluded. General characteristics of study participants are presented in **Table 2**.

2.2 Data Collection and Supplementation Protocol

Following baseline blood sample collection, participants received a daily oral dose of 500 mg of vitamin C for 30 days, taken after a meal. After supplementation, a second blood sample was obtained. A structured questionnaire was administered to collect demographic and health-related information.

2.3 Blood Sample Collection

Using a simple random sampling technique, approximately 5 mL of venous blood was collected from each participant using serum-separating gel tubes (SST). Samples were collected eight hours after the last Narghile session, from 9 a.m. to 11 a.m., allowed to clot for 20 minutes, and then centrifuged at 2000–2500 rpm for 15 minutes to isolate serum for biochemical analyses. All blood samples were preserved in deep freezing conditions (–20 °C) under standardized conditions for subsequent assays to minimize pre-analytical variability.

2.4 Measurement of Oxidative Stress and Antioxidant Biomarkers

2.4.1 Serum 8-Hydroxy-2'-deoxyguanosine (8-OHdG)

Serum 8-OHdG levels were determined using a commercial ELISA kit (SL2044Hu), employing the sandwich ELISA method. Samples and standards were added to pre-coated wells, followed by HRP-conjugated antibodies, substrate incubation, and colorimetric detection at 450 nm. Strict adherence to the manufacturer's instructions was maintained, including temperature control (2–8°C), prevention of light exposure, and avoiding cross-contamination or freeze-thaw cycles. The standard curve (Figure 1) demonstrated a strong linear correlation between 8-OHdG concentrations and optical density values, indicating assay accuracy and reliability.

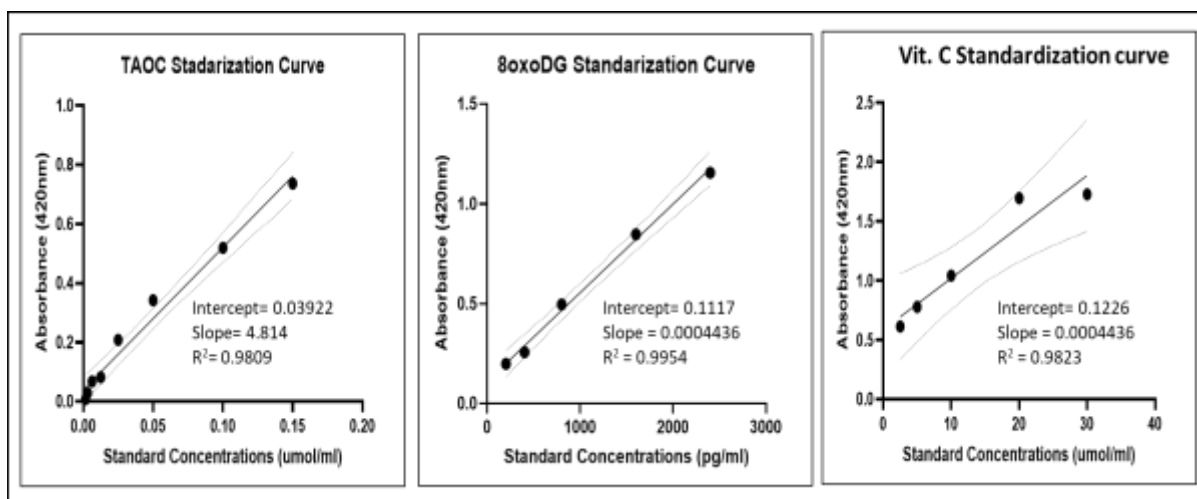


Fig. 1. Standard curve set for TAOC, 8oxoDG, and serum Vitamin C, respectively, by the ELISA kits. Standard concentrations were prepared according to the Kit protocol. Data were plotted using Prism Graph Pad software.

2.4.2 Total Antioxidant Capacity (TAOC)

TAOC was assessed using the BC1315 assay kit, which quantifies antioxidant capacity based on the reduction of Fe^{3+} -TPTZ to the blue Fe^{2+} -TPTZ complex. Absorbance was measured at 593 nm, and concentrations were calculated using a standard curve created from Fe^{2+} standards [16]. Figure 1 illustrates the strong linear relationship between absorbance and antioxidant concentration.

2.4.3 Serum Vitamin C

Serum vitamin C levels were quantified using an ELISA kit (SL1829Hu) based on a sandwich immunoassay format. The assay utilized HRP-conjugated detection antibodies and colorimetric detection at 450 nm. The kit's sensitivity is 0.1 ng/mL with a working range of 0.4–30 ng/mL. Figure 1 presents the standard curve demonstrating a robust linear relationship between concentration and absorbance, enabling accurate quantification of serum vitamin C levels.

2.4.4 Serum Malondialdehyde (MDA)

Serum MDA was measured by the method of Binge and Aust (1978). Spectrophotometrically, MDA levels in the samples were estimated using a TBA solution. In brief to 0.5 ml of serum sample added the followings: 1 ml of TBA reagent (0.375 gm TBA, 15 gm TCA, in 100 ml 0.25 N HCl) was added and mixed well by vortex, incubated in the boiling water for 1 hour at 95 °C then allow the mixture to cool, then let the mixture to stand at room temperature for 20 minutes, centrifuged at 3000 rpm for 15 minutes and take out the supernatant for spectrophotometrically estimation. The same steps were used to make the blank, but in the first step, D.W. is used instead of serum. At 532 nm, the absorbance of each pink-colored supernatant solution was measured and compared with a blank to calculate MDA levels.

MDA levels were estimated using the following formula:

$$\text{MDA (nmol/L)} = (\text{Test O.D} - \text{Blank O.D}) / \text{E}^\circ\text{L} \times 10^6$$

$$\text{Molar extinction coefficient} = 1.56 \times 10^5 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$$

$$\text{L (path length)} = 1 \text{ cm}$$

2.5 Data Analysis

All data are expressed as mean $\pm$ standard deviation (SD). Statistical analyses were conducted using GraphPad Prism (version 10.5.0). For within-group comparisons (pre- and post-supplementation), paired t-tests were used. Between-group comparisons were analyzed using unpaired t-tests. A p-value of ≤ 0.05 was considered statistically significant. Also, Data for statistical analysis were entered and validated via Microsoft Excel.

3. RESULTS

3.1 Comparison of Oxidative Stress Markers Between Narghile Smokers and Non-Smokers

The assessment of oxidative stress biomarkers 8-OHdG, MDA, TAOC, and serum vitamin C revealed significant biochemical differences between Narghile smokers and non-smokers.

Serum MDA levels were markedly elevated in Narghile smokers compared to non-smokers across both genders. The mean $\pm$ SD values were $1.390 \pm 0.3927 \mu\text{mol/L}$ in smokers versus $0.8238 \pm 0.2581 \mu\text{mol/L}$ in non-smokers ($p < 0.0001$) (Fig. 2A), indicating increased lipid peroxidation in the smoking group. Conversely, TAOC levels, a marker of overall antioxidant defense, did not significantly differ between the two groups. Smokers had a mean $\pm$ SD of $0.09151 \pm 0.01553 \mu\text{mol/mL}$, whereas non-smokers showed $0.09404 \pm 0.01260 \mu\text{mol/mL}$ ($p = 0.3522$) (Fig. 2B), a relatively preserved antioxidant capacity despite exposure. Serum 8-OHdG, a marker of oxidative DNA damage, was slightly elevated in smokers ($893.6 \pm 137.3 \text{ pg/mL}$) compared to non-smokers ($860.2 \pm 118.7 \text{ pg/mL}$), though the difference was not statistically significant ($p = 0.2138$) (Fig. 2C). However, vitamin C levels were significantly lower in Narghile smokers ($14.16 \pm 1.988 \mu\text{mol/mL}$) compared to non-smokers (16.66 ± 3.935

μmol/mL), with a statistically significant difference ($p = 0.0082$) (Fig. 2D), reflecting depletion of this antioxidant in response to oxidative stress.

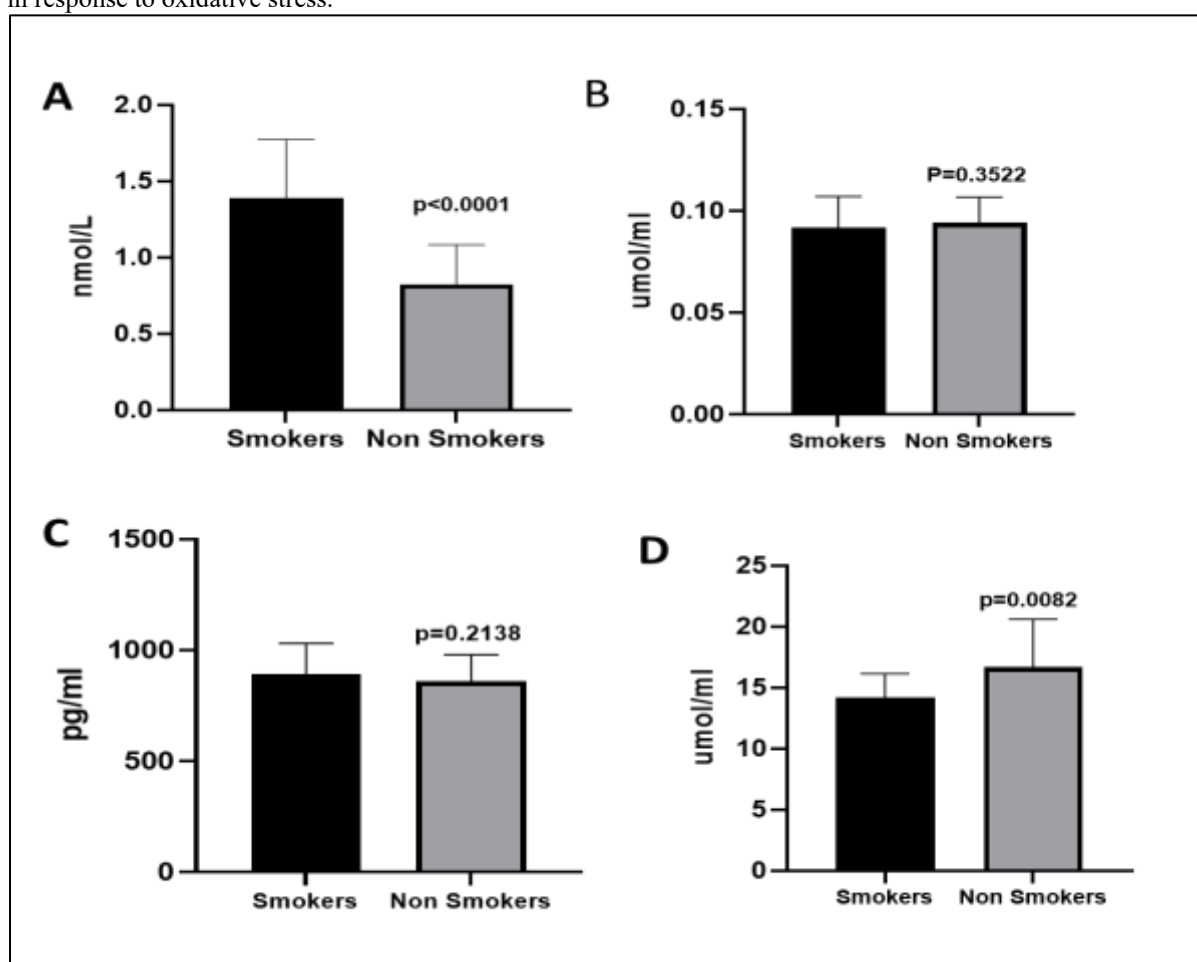


Fig. 2. OS markers and serum vitamin C level in Narghile smokers and nonsmokers. A: MDA level between all smokers and non-smokers. B: TAOC Between all smokers and non-smokers. C: 8oxodG between all smokers and non-smokers. D: Vit. C-level between all smokers and non-smokers.

3.2 Analysis by Gender

Additional analyses were conducted to evaluate differences in serum oxidative biomarkers, total antioxidant capacity (TAOC), and vitamin C levels between male and female Narghile smokers and non-smokers.

In male Narghile smokers, serum malondialdehyde (MDA) levels were significantly higher compared to non-smoker males (Mean $\pm$ SD: 1.550 ± 0.33 nmol/L vs. 0.86 ± 0.19 nmol/L, $p < 0.0001$). Other parameters, including serum TAOC and 8-oxodG, were slightly elevated in male smokers compared to non-smoker males, although these differences were not statistically significant. Notably, serum vitamin C levels were significantly lower in male smokers (13.45 ± 1.95 μmol/mL) than in non-smoker males (18.26 ± 4.754 μmol/mL, $p = 0.0116$). In contrast, no significant differences in serum MDA, TAOC, 8-oxodG, or vitamin C were observed between female Narghile smokers and non-smokers (**Table I**).

TABLE I. OS BIOMARKERS (MEAN $\pm$ SE; P-VALUE) IN BOTH GROUPS OF SMOKERS AND NONSMOKERS AMONG BOTH GENDERS OF PARTICIPANTS.

Biochemicals	Mean $\pm$ SD		P Value
	Smokers (Males)	Non smokers (Males)	
Serum MDA (nmol/L)	1.55 $\pm$ 0.33	0.86 $\pm$ 0.19	<0.0001*** ¹
Serum TAOC (μmol/ml)	0.11 $\pm$ 0.11	0.101 $\pm$ 0.12	0.22
Serum 8-OHdG (pg/ml)	929.50 $\pm$ 11.78	915.7 $\pm$ 13.51	0.72

¹***and * $p < 0.05$ indicates statistical significance

Serum Vit. C (μmol/ml)	13.45±1.95	18.26 ±4.75	0.0116*
Biochemicals	Mean ± SD		P Value
	Smokers (Females)	Non smokers (Females)	
Serum MDA (nmol/L)	0.78±0.32	0.81±0.33	0.71
Serum TAOC (μmol/ml)	0.087±0.35	0.085±0.13	0.13
Serum 8-OHdG (pg/ml)	815.80±8.62	863.0±15.08	0.16
Serum Vit. C (μmol/ml)	14.80±0.19	15.05 ±0.21	0.77

Further gender-specific comparisons among Narghile smokers revealed that males exhibited significantly higher serum MDA levels than females (1.550 ± 0.324 vs. 0.9015 ± 0.173 nmol/L, $p < 0.0001$) (Fig. 3A). Similarly, male smokers showed significantly elevated serum TAOC compared to female smokers (0.1070 ± 0.016 vs. 0.0852 ± 0.010 μmol/mL, $p < 0.0001$) (Fig. 3B). Serum 8-oxodG levels were higher in male smokers than female smokers (929.5 ± 116.0 vs. 863.0 ± 148.5 pg/mL), although this difference did not reach statistical significance ($p = 0.0965$) (Fig. 3C). Serum vitamin C concentration tended to be lower in male smokers compared to female smokers (13.45 ± 1.925 vs. 14.80 ± 1.880 μmol/mL), but this difference was not statistically significant ($p = 0.0902$) (Fig. 3D).

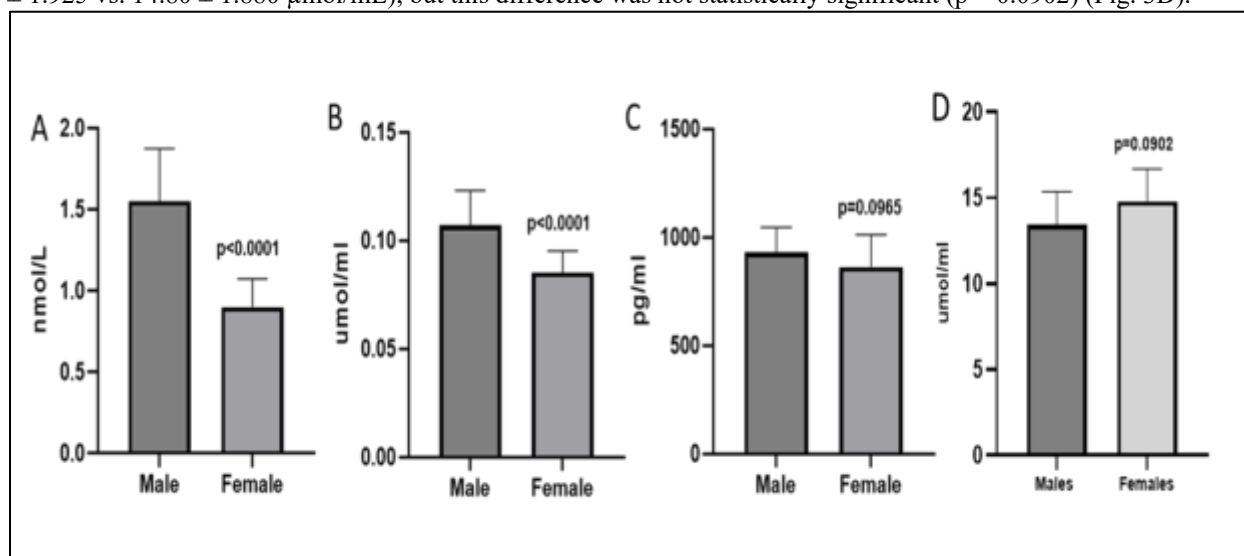


Fig. 3. OS markers and serum vitamin C level among both genders Narghile Smokers (A: MDA, B: TAOC, C: 8-OHdG, D: serum Vit. C-level concentration).

3.3 Effect of Vitamin C Supplementation on Oxidative Biomarkers

To investigate the prooxidant or antioxidant effects of vitamin C on oxidative stress markers, serum levels of malondialdehyde (MDA), 8-hydroxy-2'-deoxyguanosine (8-oxodG), and total antioxidant capacity (TAOC) were measured in Narghile smokers and non-smokers before and after one month of oral vitamin C supplementation (400 mg/day).

Notably, Narghile smokers exhibited significant changes in oxidative biomarkers following vitamin C administration. Serum MDA levels decreased significantly from 1.390 ± 0.3827 nmol/L before supplementation to 1.017 ± 0.2480 nmol/L after supplementation ($p = 0.0001$) (Fig. 4A). Concurrently, serum TAOC increased significantly in smokers post-supplementation, with mean values rising from 0.09452 ± 0.01494 μmol/mL to 0.09987 ± 0.01708 μmol/mL ($p = 0.0237$) (Fig. 4B).

Interestingly, serum 8-oxodG levels increased significantly after vitamin C supplementation in smokers, from 881.2 ± 133.0 pg/mL to 1013 ± 134.8 pg/mL ($p < 0.0001$) (Fig. 4C), suggesting a potential prooxidant effect under these conditions.

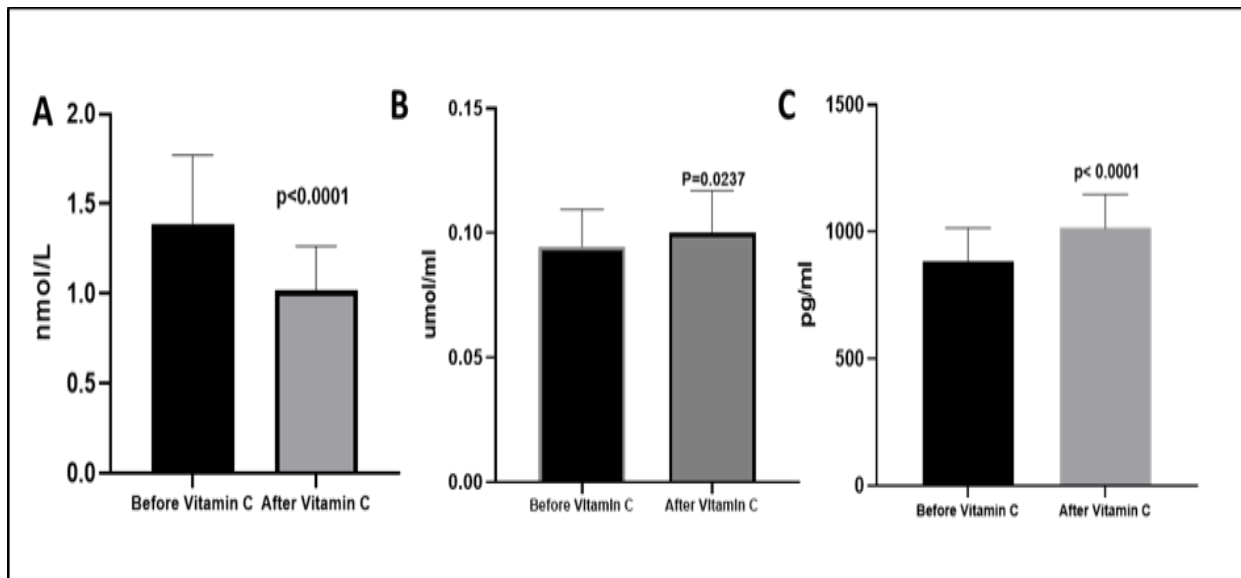


Fig. 4. Level of MDA, TAOC, and 8-OHdG before and after vitamin C supplementation (A: MDA, B: TAOC, C: 8-OHdG) in Narghile Smokers.

3.4 Gender-Specific Effects of Vitamin C Supplementation on Oxidative Biomarkers in Narghile Smokers

Serum levels of oxidative biomarkers—including malondialdehyde (MDA), 8-hydroxy-2'-deoxyguanosine (8-oxodG), and total antioxidant capacity (TAOC)—were analyzed in male and female Narghile smokers before and after one month of vitamin C supplementation.

In female smokers, serum MDA levels decreased significantly from 1.259 ± 0.383 nmol/L to 0.902 ± 0.173 nmol/L following supplementation ($p < 0.0001$) (Fig. 5A). However, no significant change was observed in serum TAOC, which remained relatively stable (0.418 ± 0.049 vs. 0.415 ± 0.061 μ mol/mL before and after supplementation, respectively; $p = 0.849$) (Fig. 5B). Notably, serum 8-oxodG levels increased significantly after supplementation, rising from 863.3 ± 148.5 pg/mL to 968.7 ± 129.1 pg/mL ($p = 0.0089$) (Fig. 5C).

Similar trends were observed in male smokers. Serum MDA significantly decreased from 1.556 ± 0.324 nmol/L to 1.158 ± 0.257 nmol/L post-supplementation ($p < 0.0001$) (Fig. 5D). Serum TAOC showed a non-significant increase from 0.1070 ± 0.016 to 0.1135 ± 0.018 μ mol/mL ($p = 0.220$) (Fig. 5E). Meanwhile, serum 8-oxodG increased significantly, from 929.5 ± 116.0 pg/mL to 1081 ± 162.7 pg/mL after vitamin C supplementation ($p = 0.0009$) (Fig. 5F).

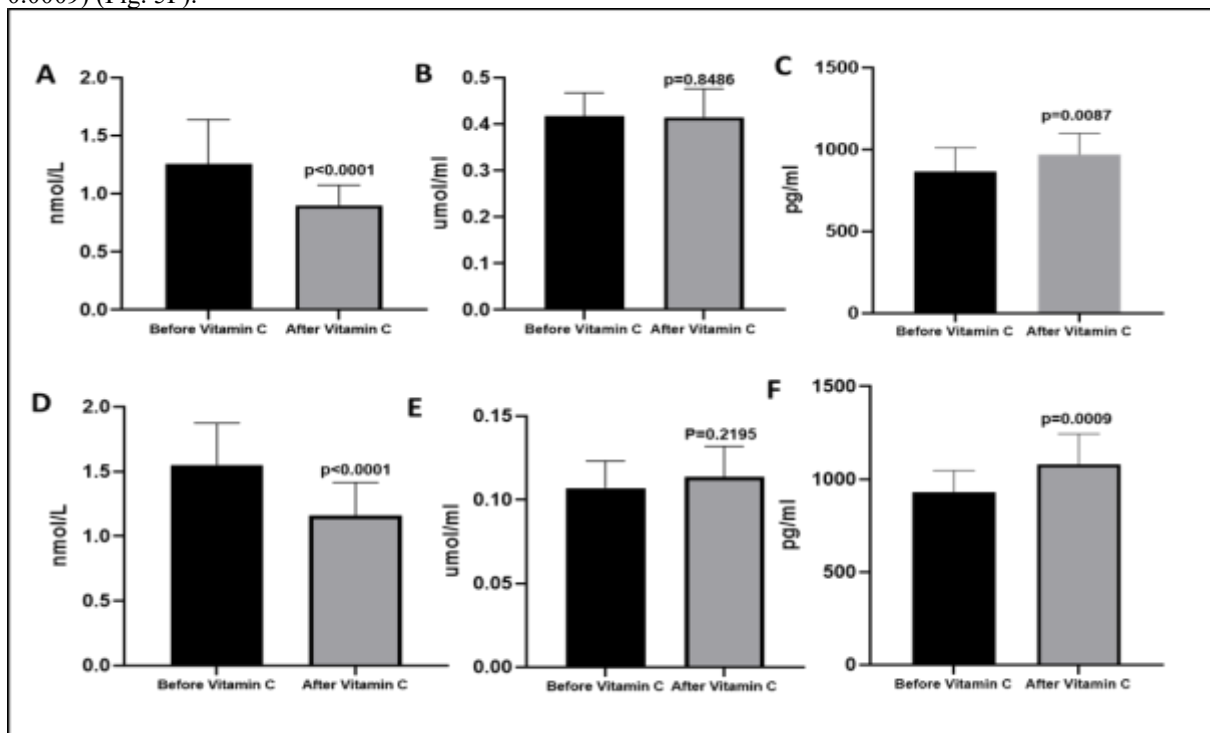


Fig. 5. MDA, TAOC, and 8-OHdG levels before and after the vitamin C supplementation in Male and Female participants. Data are presented as (mean $\pm$ SE; p-value). (A: MDA level B: TAQ C: 8-OHdG) in Females. (D: MDA E: TAOC F: 8-OHdG) in Males.

3.5 Demographic and Clinical Profiles of Participants

Across the overall study population, comparisons between Narghile smokers and nonsmokers revealed notable demographic differences. The average age of Narghile smokers was 22.57 ± 4.20 years, while nonsmokers averaged 24.31 ± 3.20 years. Although smokers tended to be younger, the difference was not statistically significant ($p = 0.09$). Regarding body mass index (BMI), smokers had a mean of 25.88 ± 4.40 , compared to 26.70 ± 6.64 in nonsmokers, with no statistically significant difference between the two groups ($p = 0.35$).

By contrast, blood pressure values highlighted more pronounced differences. Smokers demonstrated a markedly higher mean systolic blood pressure (125.51 ± 1.15 mmHg) compared with nonsmokers (109.78 ± 1.18 mmHg). Although the difference did not achieve statistical significance ($p = 0.36$), the numerical trend suggests a possible impact of smoking on systolic pressure. Similarly, diastolic blood pressure was elevated in smokers (85.01 ± 1.54 mmHg) compared with nonsmokers (75.33 ± 0.97 mmHg), with the p -value approaching significance ($p = 0.06$). Participants in the study are categorized by their demographic and baseline characteristics, as shown in Table II.

TABLE II. GENERAL CHARACTERISTICS OF NARGHILE SMOKERS AND NON-SMOKERS (MEAN $\pm$ SD)

Parameter	Group	Mean $\pm$ SD	P-Value
Age	Smoker	22.571 $\pm$ 4.2032	0.09
	Nonsmokers	24.311 $\pm$ 3.1967	
BMI	Smokers	25.877 $\pm$ 4.3999	0.3483
	Nonsmokers	26.695 $\pm$ 6.6361	
Systolic	Smokers	125.51 $\pm$ 1.149	0.3583
	Nonsmokers	109.78 $\pm$ 1.1830	
Diastolic	Smokers	85.012 $\pm$ 1.5408	0.061
	Nonsmokers	75.326 $\pm$ 0.9673	

4. DISCUSSION

Narghile smoking introduces a high amount of (ROS) into the human body. It diminishes the body's natural antioxidant defenses, thereby increasing oxidative stress and enhancing damage to cellular components, including lipids, proteins, and DNA.¹² This imbalance between oxidants and antioxidants raises the risk of developing serious health conditions among Narghile smokers.¹³

The antioxidant defense system in the human body, comprising enzymatic and non-enzymatic components, helps maintain redox balance following ROS production during metabolism.¹⁴ Vitamin C is an important exogenous antioxidant found in various vegetables and fruits. It plays a crucial role in scavenging free radicals.¹⁵ However, under certain conditions, such as high concentrations or in the presence of transition metal ions (iron or copper), vitamin C may behave as a prooxidant. It reacts with hydrogen peroxide (H₂O₂) to produce hydroxyl radicals ($\cdot$ OH) through Fenton-type reactions, thereby promoting further oxidative stress.¹⁶

In this study, we assessed oxidative status in Narghile smokers and non-smokers by measuring serum oxidative biomarkers (MDA and 8-oxoDG), TAOC, and vitamin C levels. To evaluate the antioxidant and/or prooxidant effects of vitamin C, we also measured these biomarkers after one month of daily vitamin C supplementation in both groups.

This study's data revealed a significantly higher level of serum MDA in Narghile smokers compared to non-smokers ($p < 0.0001$), indicating an elevated level of lipid peroxidation and oxidative stress among smokers. Similar findings have been documented in earlier studies.¹⁷ The elevated ROS and free radicals, mainly originating from the combustion of charcoal and tobacco, contribute to increased oxidative DNA damage in Narghile smokers. These observations are consistent with previous studies reporting heightened oxidative stress in Narghile users.¹⁸ Whereas serum TAOC and 8-oxoDG levels did not differ significantly between smokers and non-smokers, serum vitamin C levels were notably lower in smokers ($p = 0.0082$). Prior research has similarly shown reduced serum vitamin C in tobacco smokers, even with comparable dietary intake.¹⁹ This reduction may result from increased consumption of vitamin C to neutralize excessive oxidants or its involvement in prooxidant reactions such as Fenton chemistry.

We further analyzed the oxidative stress profile by gender. Male Narghile smokers had significantly elevated serum MDA levels compared to male non-smokers ($p < 0.0001$). Female smokers showed a non-significant increase in MDA levels compared to female non-smokers ($p = 0.7726$), possibly due to the protective antioxidant

properties of estrogen (Christine et al., 2017). In males, serum vitamin C was significantly lower in smokers than in non-smokers ($p = 0.00116$), suggesting higher ascorbate utilization in response to oxidative stress. Comparisons between male and female smokers revealed higher MDA levels in males ($p < 0.0001$) and lower TAOC levels in females ($p < 0.0001$), suggesting gender-related differences in oxidative stress response. Additionally, serum 8-oxoDG was slightly higher in males ($p = 0.0965$), whereas vitamin C was lower in males compared to females ($p = 0.0902$).

A marked change in all serum biomarkers was observed in Narghile smokers following one month of vitamin C supplementation. Specifically, vitamin C significantly reduced lipid peroxidation, as indicated by decreased MDA levels ($p < 0.0001$, Fig. 4A). It also increased TAOC levels in smokers ($p = 0.0237$, Fig. 4B). However, an unexpected finding was the significant increase in serum 8-oxoDG after vitamin C supplementation ($p < 0.0001$, Fig. 4C), indicating enhanced oxidative DNA damage. This may be due to vitamin C acting as a prooxidant under specific intracellular conditions, particularly in the presence of metal ions that promote Fenton-type reactions.²⁰ Serum vitamin C levels were significantly higher in non-smokers compared to Narghile smokers ($p = 0.0082$), possibly reflecting compensatory utilization of ascorbate in response to smoking-induced oxidative stress.²¹ These findings highlight the long-term molecular consequences of Narghile smoking on cellular components, particularly lipids and DNA. The elevated 8-oxoDG levels following vitamin C supplementation in smokers provide insight into the dual role of vitamin C, as both antioxidant and prooxidant, potentially contributing to mutagenesis, inflammation, and disease progression, including cancer and cardiovascular disorders.

A major strength of this study is its biomarker-based approach, which provides a measurable assessment of oxidative stress and the dual antioxidant/prooxidant nature of vitamin C in Narghile smokers and non-smokers. This study has a limitation of the sample size, which was relatively small (50 smokers and 47 non-smokers). This may limit the statistical power and generalizability of the findings.

In summary, the study results contribute to the growing evidence that Narghile smoking induces oxidative stress, particularly at the DNA level. Furthermore, vitamin C may exacerbate DNA oxidative damage in smokers, highlighting its potential prooxidant role in specific contexts. These findings warrant careful consideration in public health recommendations, particularly concerning vitamin C supplementation among smokers.

5. CONCLUSION

A novel investigation was conducted based on the results of this research. Although serum MDA levels decreased in Narghile smokers following vitamin C supplementation, this change may reflect complex alterations in oxidative metabolism rather than a straightforward protective effect. The findings provide clear evidence that Narghile smoking is associated with increased oxidative stress, particularly demonstrated by a significant rise in serum 8-OHdG levels after one month of vitamin C intake, an indicator of enhanced oxidative DNA damage. In contrast, TAOC remained unchanged, suggesting that systemic antioxidant defenses may not be immediately affected during the early stages of exposure to vitamin C supplementation.

These results highlight that, whereas overall antioxidant capacity may initially appear stable, Narghile smoking imposes substantial oxidative DNA damage at the cellular level. This underscores the need for increased awareness regarding the molecular and cellular health risks associated with Narghile smoking, particularly its impact on DNA integrity.

REFERENCES

1. Asiedua C, Abah E, Asare M. Theoretical Perspectives on Hookah Smoking Behavior: A Scoping Review of Studies Among Young Adults in Low- and Middle-Income Countries. *Journal of community health*. 2025;50(761–780):10.1007/s10900-02501449-7. doi:https://doi.org/10.1007/s10900-025-01449-7
2. Al-Kalbani S. The Characteristics of Shisha Smokers in Oman: A Cross-Sectional Study. *Frontiers*. 2025;5(1):53–61. doi:https://doi.org/10.11648/j.frontiers.20250501.14
3. Mahmood DK, Sehrash Afreen, Khan HM, Rahim W. Side Effects of Using Shisha on the Human's Health: A Systematic Review. *Journal of Political Stability Archive*. 2024;2(4):36–53. Accessed September 15, 2025. https://journalpsa.com.pk/index.php/JPSA/article/view/25
4. Haldar D, Mallik S, Sarkar G, Das S, Lahiri S, Chatterjee T. A study on habits of tobacco use among medical and non-medical students of Kolkata. *Lung India*. 2011;28(1):5. doi:https://doi.org/10.4103/0970-2113.76293
5. Hishyar Azo Najeeb, Karim KK, Othman GO, Mohammed AA, Jamel D. DNA damage level in lymphocytes and buccal epithelial cells in Narghile smokers and non-smokers in Duhok city-KRG-Iraq. *Oral Oncology Reports*. 2022;1-2(80). doi:https://doi.org/10.1016/j.oor.2022.100002
6. Jebai R, Ebrahimi Kalan M, Vargas-Rivera M, et al. Markers of oxidative stress and toxicant exposure among young waterpipe smokers in the USA. *Environmental Science and Pollution Research*. 2021;28(21):26677–26683. doi:https://doi.org/10.1007/s11356-021-12520-w
7. Najeeb HA, Sanusi T, Saldanha G, Brown K, Cooke MS, George DD. Jones. Redox modulation of oxidatively-induced DNA damage by ascorbate enhances both in vitro and ex-vivo DNA damage formation and cell death in melanoma cells. *Free radical biology & medicine*. 2024;213:309–321. doi:https://doi.org/10.1016/j.freeradbiomed.2024.01.019
8. Badran M, Laher I. Waterpipe (shisha, hookah) smoking, oxidative stress and hidden disease potential. *Redox Biology*. 2020;34:101455. doi:https://doi.org/10.1016/j.redox.2020.101455

9. Alameri AA. Assessing the Impact of Hookah Smoking on Oxidative Stress Markers in Young Adults: A Cross-Sectional Study. Scione.com. Published 2024. Accessed September 15, 2025. <https://asb.scione.com/cms/fulltext.php?id=200>
10. Taati B, Arazi H, Suzuki K. Oxidative Stress and Inflammation Induced by Waterpipe Tobacco Smoking Despite Possible Protective Effects of Exercise Training: A Review of the Literature. *Antioxidants*. 2020;9(9):777. doi:<https://doi.org/10.3390/antiox9090777>
11. Kamil W, Zahraa Hussein M.Kadri. Evaluation of Malondialdehyde, C-reactive Protein and DNA Damage Related with the Smoking Habit by Comet... *International Journal of Drug Delivery Technology*. 2021;11(2):298-302. doi:<https://doi.org/10.25258/ijddt.11.2.10>
12. Mohammed A, Farouk D, Adelf Hashish. Relationship between oxidative stress biomarkers and micronuclei scoring as a biomarker for DNA damage in Egyptian nicotine-dependent smokers: A cross-sectional study. *Journal of The Arab Society for Medical Research*. 2023;18(2):161. doi:https://doi.org/10.4103/jasmr.jasmr_8_23
13. Kaźmierczak-Barańska J, Boguszevska K, Adamus-Grabicka A, Karwowski BT. Two Faces of Vitamin C—Antioxidative and Pro-Oxidative Agent. *Nutrients*. 2020;12(5):1501. doi:<https://doi.org/10.3390/nu12051501>
14. Seo YS, Jung Min Park, Kim JH, Lee MY. Cigarette Smoke-Induced Reactive Oxygen Species Formation: A Concise Review. *Antioxidants*. 2023;12(9):1732-1732. doi:<https://doi.org/10.3390/antiox12091732>
15. Mahdi Sepidarkish, Shima Rezazadeh, Helia Ghaffari Hamedani, et al. The waterpipe smoking and human health: a systematic review and meta-analysis of 191 observational studies. *Systematic Reviews*. 2025;14(1). doi:<https://doi.org/10.1186/s13643-025-02799-y>
16. Wayner DDM, Burton GW, Ingold KU, Locke S. Quantitative measurement of the total, peroxyl radical-trapping antioxidant capability of human blood plasma by controlled peroxidation. *FEBS Letters*. 1985;187(1):33-37. doi:[https://doi.org/10.1016/0014-5793\(85\)81208-4](https://doi.org/10.1016/0014-5793(85)81208-4)
17. Rianganapatee. Effect of surfactant on lycopene-loaded nanostructured lipid carriers. *Drug Discoveries & Therapeutics*. 2012;6.3(163). doi:<https://doi.org/10.5582/ddt.2012.v6.3.163>
18. Chatgililoglu C. Biomarkers of Oxidative and Radical Stress. *Biomolecules*. 2024;14(2):194. doi:<https://doi.org/10.3390/biom14020194>
19. Krishnamurthy HK, Pereira M, Imbaasree Rajavelu, et al. Oxidative stress: fundamentals and advances in quantification techniques. *Frontiers in Chemistry*. 2024;12. doi:<https://doi.org/10.3389/fchem.2024.1470458>
20. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative Stress and Antioxidant Defense. *World Allergy Organization Journal*. 2012;5(1):9-19. doi:<https://doi.org/10.1097/wox.0b013e3182439613>
21. Sharma Y, Subodha Sumanadasa, Shahi R, et al. Efficacy and safety of vitamin C supplementation in the treatment of community-acquired pneumonia: a systematic review and meta-analysis with trial sequential analysis. *Scientific Reports*. 2024;14(1). doi:<https://doi.org/10.1038/s41598-024-62571-5>
22. Dhia AL-Timimi, MOHAMMAD. ZINC STATUS AMONG SMOKERS AND NON SMOKERS: RELATION TO OXIDATIVE STRESS. *Duhok Medical Journal*. 2010;4(1):67. https://www.researchgate.net/publication/278218946_ZINC_STATUS_AMONG_SMOKERS_AND_NON_SMOKERS_RELATION_TO_OXIDATIVE_STRESS
23. SMITH JL, HODGES RE. Serum Levels of Vitamin C in Relation to Dietary and Supplemental Intake of Vitamin C in Smokers and Nonsmokers. *Annals of the New York Academy of Sciences*. 1987;498(1 Third Confere):144-152. doi:<https://doi.org/10.1111/j.1749-6632.1987.tb23758.x>
24. Kander MC, Cui Y, Liu Z. Gender difference in oxidative stress: a new look at the mechanisms for cardiovascular diseases. *Journal of Cellular and Molecular Medicine*. 2017;21(5):1024-1032. doi:<https://doi.org/10.1111/jcmm.13038>
26. Chiorcea-Paquim AM. 8-oxoguanine and 8-oxodeoxyguanosine Biomarkers of Oxidative DNA Damage: A Review on HPLC–ECD Determination. *Molecules*. 2022;27(5):1620. doi: <https://doi.org/10.3390/molecules27051620>
27. Dehghan M, Akhtar-Danesh N, McMillan CR, Thabane L. Is plasma vitamin C an appropriate biomarker of vitamin C intake? A systematic review and meta-analysis. *Nutrition Journal*. 2007;6(1). doi: <https://doi.org/10.1186/1475-2891-6-41>