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# The Effect of Cigarette Smoke Exposure on Blood Nicotine and Glutathione (GSH) Levels in Smokers and Non-Smokers

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## ABSTRACT

Smoking remains a major global public health problem and contributes substantially to morbidity and mortality worldwide. According to the World Health Organization, tobacco use causes more than 8 million deaths annually, including approximately 1.3 million deaths among non-smokers exposed to secondhand smoke. Cigarette smoke contains nicotine and various oxidant compounds that promote free radical formation and oxidative stress, potentially disrupting the antioxidant defense system, including reduced glutathione (GSH), an important intracellular antioxidant. However, evidence simultaneously comparing nicotine exposure and GSH levels between smokers and non-smokers remains limited, particularly in the studied population. This study aimed to compare blood nicotine and GSH levels between smokers and non-smokers. An observational analytical study with a cross-sectional design was conducted among 34 respondents, comprising 17 smokers and 17 non-smokers. EDTA plasma samples were analyzed using UV–Vis spectrophotometry at 258 nm for nicotine and 412 nm for GSH. Differences between groups were analyzed using the Mann–Whitney U test. The mean nicotine level was higher in smokers than in non-smokers (19.18 vs. 5.51 µg/mL), whereas the mean GSH level was lower in smokers than in non-smokers (27.00 vs. 38.82 mg/dL). Significant differences were observed in both nicotine and GSH levels between the two groups ($p < 0.001$). These findings indicate that smoking is associated with higher nicotine levels and lower GSH levels, suggesting an imbalance in antioxidant defense consistent with increased oxidative stress among smokers.

Keywords: Smoker; Non-Smoker; Nicotine; Glutathione; UV-Vis Spectrophotometer

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## INTRODUCTION

Smoking remains a major public health concern worldwide, particularly in Indonesia, where tobacco consumption remains high. Indonesia has one of the largest populations of smokers globally and ranks among the countries with the highest cigarette consumption (2). National data indicate a substantial increase in the number of adult smokers, from 60.3 million in 2011 to 69.1 million in 2021 (3). These figures highlight the persistent burden of tobacco use and the potential health risks associated with both active smoking and secondhand smoke exposure. These adverse health effects are associated with exposure to nicotine, oxidants, and other toxic compounds in cigarette smoke that may contribute to various biological disturbances, including oxidative stress.

Cigarette smoke exposure may occur directly through active smoking or indirectly through secondhand smoke. Active smokers directly inhale smoke generated during cigarette consumption, whereas non-smokers may be involuntarily exposed to cigarette smoke in their surrounding environment (4)). Cigarette smoke contains numerous biologically active and potentially toxic compounds, including nicotine (5). Nicotine is a naturally occurring alkaloid in tobacco that is released during tobacco combustion and rapidly absorbed through the respiratory tract into the systemic circulation. After absorption, nicotine is extensively metabolized in the liver, predominantly by cytochrome P450 2A6 (CYP2A6), into cotinine and other metabolites (11). Therefore, the measurement of nicotine in biological samples may provide information regarding exposure to tobacco-derived compounds.

In addition to nicotine, cigarette smoke contains oxidants and free radicals that can promote the formation of reactive oxygen species (ROS). Excessive ROS production may disrupt the balance between oxidants and endogenous antioxidant defenses, resulting in oxidative stress and subsequent damage to cellular components, including lipids, proteins, and DNA. Glutathione (GSH) is an important non-enzymatic intracellular antioxidant that plays a central role in maintaining cellular redox homeostasis and protecting cells against oxidative damage. GSH participates in the neutralization of reactive species and serves as a substrate for antioxidant enzymes, including glutathione peroxidase (GPx). Persistent exposure to cigarette smoke-derived oxidants may increase the utilization of GSH in antioxidant defense, potentially contributing to GSH depletion and reduced antioxidant capacity (12,23). Thus, nicotine levels may reflect tobacco-related exposure, whereas GSH levels may provide complementary information regarding antioxidant defense in response to oxidative stress.

Nicotine is a natural alkaloid compound found in tobacco leaves. In conventional cigarettes, nicotine is absorbed through the smoke resulting from burning tobacco, while in vape nicotine is absorbed through steam produced from heating e-liquid. The nicotine content in cigarettes can cause addiction, making it difficult to stop smoking. As much as 80% of nicotine is converted into cotinine by the CYP2A6 enzyme in the liver and excreted in the urine (6). Nicotine consumption can cause inflammation of the lungs and respiratory tract, increase reactive oxygen species (ROS) endogenously and exogenously, damage cells and blood vessels and cause coronary heart disease. Glutathione

peroxidase or GSH is an enzyme that produces glutathione peroxidation. Peroxidase can occur due to exposure to free radicals both from pollutants and bacterial and viral infections. Free radicals from cigarette smoke cause peroxidation of polyunsaturated fatty acids in cells, exacerbating oxidative stress (23). The decrease in glutathione peroxidase (GSH) levels is related to the effects of free radicals from cigarette smoke which cause oxidative stress (7).

Based on previous studies by (8), there is a relationship between high levels of nicotine in smokers' blood and lipid profiles. Research using a UV-Vis spectrophotometer shows that nicotine levels in smokers are between 11-52 µg/ml, while in non-smokers it is 0.9-9.7 µg/ml, indicating a close relationship between serum cholesterol and HDL levels with smoking. Another study by (22) using GC-MS (Gas Chromatography-Mass Spectrometry) showed the highest nicotine levels were 4,778 mg/ml in active smokers and 1,936 mg/ml in passive smokers. (24) research using HPLC (High Performance Liquid Chromatography) shows an average nicotine level of 0.10 ppm in active smokers, which correlates with triglyceride levels in the blood of active smokers. Research by (25) shows that plasma GSH levels of inorganic farmers are lower than those of organic farmers. Research by (1) on gas station workers showed a decrease in GSH levels, although not significant. Research by (9) in COPD patients showed that allopurinol administration can reduce GSH levels.

Previous studies have reported differences in nicotine concentrations according to patterns of cigarette smoke exposure and associations between smoking-related exposure and alterations in biochemical parameters (10–12). Other studies involving populations exposed to environmental or occupational oxidative stressors have also demonstrated alterations in GSH levels, suggesting that prolonged exposure to oxidants may affect endogenous antioxidant status (13). However, previous studies have generally assessed nicotine exposure and antioxidant status separately or have focused on populations with different exposure characteristics. Evidence simultaneously comparing nicotine levels as an indicator of tobacco-related exposure and GSH levels as an indicator of antioxidant defense between smokers and non-smokers remains limited, particularly in the studied population. Simultaneous assessment of these two parameters may therefore provide a more integrated understanding of tobacco-related exposure and its association with alterations in antioxidant defense.

Therefore, this study aimed to compare blood nicotine and glutathione (GSH) levels between smokers and non-smokers using UV–Vis spectrophotometry. By simultaneously evaluating these two biomarkers, this study seeks to provide a more comprehensive understanding of the association between cigarette smoke exposure and antioxidant defense status. The study was conducted in Surabaya using blood samples obtained from 34 participants, consisting of 17 smokers with a minimum smoking history of one year and 17 non-smokers. Nicotine and glutathione (GSH) levels were determined using UV–Vis spectrophotometry.

This study employed an observational analytical design with a quantitative approach and a cross-sectional framework. In this design, the researchers did not administer any intervention or manipulate

the exposure variables but only observed and compared blood nicotine and glutathione (GSH) levels between smokers and non-smokers at a single point in time (26). Laboratory analysis of blood samples using UV–Vis spectrophotometry was performed solely to quantify nicotine and GSH concentrations and did not constitute an experimental intervention.

## METODS

### 2.1 Materials

The materials to be used in this research are venous blood sample, purified GSH, 0.1M phosphate buffer pH 8, 0.1M phosphate buffer pH 7, 5% TCA (Trichloroacetic Acid), DNTB (5,5'-Ditio-bis (2-nitrobenzoic acid), nicotine standard, $KH_2PO_4$, NaOH, HCl, pH paper, distilled water, and methanol.

### 2.2 Study Population and Sampling

Participants were recruited through purposive sampling, based on predefined inclusion and exclusion criteria.

#### 2.2.1 Inclusion and Exclusion criteria

Inclusion criteria

For the smoker group, participants were required to:

A. Be willing to participate in the study by providing written informed consent.
B. Be male or female.
C. Reside in Surabaya, Indonesia.
D. Have smoked cigarettes regularly for at least one year.
E. Use conventional filter cigarettes, non-filter cigarettes, or electronic cigarettes.
F. Be between 18 and 50 years of age.

For the non-smoker group, participants were required to:

A. Be willing to participate in the study by providing written informed consent.
B. Be male or female.
C. Have not smoked any tobacco products for at least one year.
D. Be between 18 and 50 years of age.
E. Be willing to undergo venous blood collection.

Exclusion criteria

Participants in the smoker group were excluded if they:

A. Declined to participate in the study.
B. Were younger than 18 years or older than 50 years.
C. Did not reside in Surabaya.
D. Had hemolyzed blood samples that were unsuitable for laboratory analysis.

Participants in the non-smoker group were excluded if they:

A. Declined to participate in the study.
B. Did not reside in Surabaya.

C. Were younger than 18 years or older than 50 years.

D. Had hemolyzed blood samples that were unsuitable for laboratory analysis.

### 2.2.2 Sample Size Determination

Sample size was determined using the single population proportion formula proposed:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

n = minimum required sample size

Z = standard normal deviate (1.96 for a 95% confidence level)

P = estimated population proportion

d = margin of error (precision)

where $n$ is the minimum required sample size, $Z$ is the standard normal deviate at the 95% confidence level (1.96), $P$ is the estimated population proportion (0.10), and $d$ is the margin of error (0.10). Based on this calculation, the minimum required sample size was 34.57, which was rounded to 34 participants. Therefore, the study included 34 respondents, consisting of 17 smokers and 17 non-smokers.

### 2.2.3 Ethical Approval

This study was approved by the Health Research Ethics Committee of Universitas Nahdlatul Ulama Surabaya (Ethical Approval No. 0037/KEPK/UNUSA/2024). Written informed consent was obtained from all participants prior to enrollment and blood collection.

### 2.3 Data collection procedures

The instrument used in this research is 3 cc syringe, tourniquet, alcohol swab, EDTA vacutainer tube, analytical balance, 100ml measuring flask, dropper pipette, spatula, stir bar, analytical balance, cuvette, Shimadzu brand UV-Vis spectrophotometer, measuring pipette, volume pipette, micropipette, blue tip, yellow tip, centrifuge, water bath, effendorf tube, test tube.

### 2.3.1 Venous sampling procedure

Identify respondents and then educate them about the description of the phlebotomy procedure. Disinfect hands and use gloves. Prepare tools and materials for taking venous blood samples. Syringe, alcohol cotton, tourniquet, and dry cotton, plaster, vacuum tube. Make sure the tourniquet is placed 3-4 inches from the crease of the elbow. The first choice of venipuncture is the median cubital vein. If the median cubital vein cannot be found, the basilic vein or cephalic vein can be used. The cephalic vein is a last resort because there is a risk of injury to the median nerve or puncture of the brachial artery. The tourniquet should not be in place for more than 1 minute. Disinfect counterclockwise at the venipuncture site, let it dry. Position the needle at a 15-30° angle to the skin surface. Insert the needle with the right hand and hold the respondent's arm with the left hand to avoid immobilization of the arm and vein. Hold the syringe with your left hand, and pull the plunger with your right hand. Once blood enters the syringe, remove the tourniquet immediately. After the syringe is full, dry cotton is placed at the puncture site, and the syringe is released slowly. Respondents were asked to hold the cotton at the stabbing site. The

blood is transferred into a vacuum tube. The wound is closed with plaster. Write the identity on the tube (27).

### 2.3.2 Plasma Sample procedure

Plasma is obtained by inserting the blood obtained into a purple cap Vacutainer tube. After that, centrifuge at 3000 Rpm for 15 minutes.

### 2.3.3 Preparation of Pure Nicotine Standard Curve

A standard nicotine solution of 1000 µg/ml was made. Dilute the stock solution with concentrations of 5, 10, 15, 20, and 25µg/ml. 200 µl of 2.5 M NaOH solution was added to 500 µl of each dilution. Centrifuge at 3000 rpm for 5 minutes. The supernatant was added with 10 ml of dichloromethane-diethyl ether mixture (1:1). Vortex for 1-2 minutes, centrifuge again at 3000 rpm for 5 minutes. The organic layer was transferred into a new tube, and 40 µl of 0.25 M HCl was added. Evaporated in a water bath at 35°c. Re-dissolved with 2 ml of mixed solution (0.2973 g $KH_2PO_4$ and 820 ml of distilled water and 180 ml of methanol). Standards were analyzed using a UV-Vis spectrophotometer λ 258 nm (28).

### 2.3.4 Measurement of sample nicotine levels

Sample 500µl of plasma was added with 200µl of 2.5M NaOH solution, centrifuged at 3000rpm for 5 minutes. The supernatant was taken, and 10ml of dichloromethane-diethyl ether (1:1) mixture was added. The sample was vortexed for 1-2 minutes. The sample was centrifuged at 3000rpm for 5 minutes. The organic layer was transferred into a new tube, and 40µl of 0.25M HCl was added. The organic phase was evaporated in a water bath at 35°c. Then it was re-dissolved with 2ml of the mixture solution (0.2973g $KH_2PO_4$ and 820ml of distilled water and 180 ml of methanol). Samples were analyzed using a UV-Vis spectrophotometer λ 258 nm (8).

### 2.3.5 GSH Reagent Preparation

GSH reagent is made from DNTB: 39.6 mg DNTB dissolved in 10 ml of 0.1 M phosphate buffer, pH 8.0, and TCA 5%: Standard TCA 25% solution diluted with distilled water until a TCA concentration of 5% is obtained.

### 2.3.6 GSH Calibration Curve Construction

Standard glutathione solution: 2mg/ml in 0.1M phosphate buffer pH 8.0. From the standard solution, 0.0µl; 0.5 µl; 10 µl; 20.0 µl; 25.0 µl and 50 µl were taken, each put into a test tube. Then 0.1 M phosphate buffer solution pH 8.0 was added to each tube so that the volume became 9 ml. One ml of 5% TCA solution was added to each tube, and shaken until homogeneous. From each tube, 4.0 ml was taken and 0.05 ml of DNTB reagent was added. The remaining solution of each tube was used as a blank. Furthermore, it was measured by the standard absorbance against the blank with a spectrophotometer at a wavelength of 412 nm. From the measurement data, a calibration curve is created by connecting the absorption value as the ordinate (y) and the concentration of the standard solution as the abscissa (X)(14) (15).

### 2.3.7 GSH Level Measurement of Samples

The concentration measurement of the samples was carried out by mixing 50 µl of plasma with 1.78 mL of 0.1 M phosphate buffer pH 8 and 0.2 ml of 5% TCA. The mixture was then centrifuged at 1500 Rpm for 5 minutes, at 4◦C. The supernatant was then added with 0.01 mL of DNTB and left for 1 hour. The mixture was then examined using UV-Vis spectrophotometry at a wavelength of 412 nm to determine the GSH levels.

### 2.3.8 Calculation of Nicotine anda GSH levels in samples

Nicotine and GSH absorbance results in the sample. The nicotine and GSH levels of the sample were obtained using a linear equation from the standard curve that had been obtained previously. The equation obtained is y = ax + b where (y) is the absorbance value on the UV Vis spectrophotometer and (x) is the concentration.

### 2.4 Data analysis

Statistical analyses were performed using IBM SPSS Statistics version 25. Continuous variables were expressed as mean ± standard deviation. Data normality was evaluated using the Shapiro–Wilk test because the sample size in each group was less than 50. Homogeneity of variance was assessed using Levene's test. Since the data were not normally distributed, differences between smokers and non-smokers were analyzed using the Mann–Whitney U test. Statistical significance was established at $p < 0.05$

## RESULTS

### Characteristics of Respondents by Age

Based on the research results, the distribution of respondents by age differed between the smoker and non-smoker groups. Data from the smokers group, most respondents (76.5%) were between 18-34 years old, with a total of 13 people. The remaining 4 people (23.5%) were in the 35-50 year age range. Meanwhile, the non-smokers group, all respondents (100%) were between 18-34 years old. There were no non-smoking respondents who were in the 35-50 year age range (16). There are significant age differences in the daily burden of mental and physical ill-health related to smoking status (17).

Table 1. Distribution of Respondents by Age

| Age (years) | Smokers | % | Non-Smoker | % |
|---|---|---|---|---|
| 18-34 | 13 | 76.5% | 17 | 100% |
| 35-50 | 4 | 23.5% | 0 | 0% |
| Amount | 17 | 100% | 17 | 100% |

### Characteristics of Respondents According to Gender

Based on the results of the research, the distribution of respondents by gender showed a striking difference between smokers and non-smokers. Among smokers, 17 respondents (100%) were male, and there were no female respondents. On the other hand, in the non-smoker group, the majority were female, namely 14 respondents (76.5%) and 4 male respondents (23.5%).

Table 2. Distribution of Respondents According to Gender

| Gender | Smokers | % | Non-Smokers | % |
|---|---|---|---|---|
| Male | 17 | 100% | 4 | 23.5% |
| Female | 0 | 0% | 13 | 76.5% |
| Amount | 17 | 100% | 17 | 100% |

**Characteristics of Respondents According to Smoking Habits**

Based on the study findings, most smokers were classified as light smokers. Of the 17 smokers, 13 participants (76.5%) reported smoking 1–10 cigarettes per day, followed by 3 participants (17.6%) who smoked 11–20 cigarettes per day (moderate smokers), and 1 participant (5.9%) who smoked more than 20 cigarettes per day (heavy smoker) (Table 3). According to the classification proposed by [Reference], smokers are categorized as light smokers (1–10 cigarettes/day), moderate smokers (11–20 cigarettes/day), and heavy smokers (>20 cigarettes/day). The predominance of light smokers in this study indicates that most participants had relatively low daily cigarette consumption.

Table 3. Distribution of Respondents *According to Smoking Habits*

| **Smoking habit** | **Number** | **%** |
|---|---|---|
| 1-10 cigarettes | 13 | 76.5% |
| 11-20 cigarettes | 3 | 17.6% |
| >20 cigarettes | 1 | 5.9% |
| Amount | 17 | 100% |

**Characteristics of Respondents According to Duration of Consuming Cigarettes**

Based on the results of the characteristics of consuming cigarettes for a long time, namely 1-5 year, were obtained by 6 respondents with a percentage (35.3%) for a long time-consume cigarettes for more than 5 years, namely as many as 11 respondents with a percentage (53%) of 17 respondents in the area Surabaya.

Table 4. Distribution of Respondents According to Duration of Consuming Cigarettes

| **Smoking duration** | **Number** | **%** |
|---|---|---|
| 1-5 years | 6 | 35.3% |
| >5 years | 11 | 64.7% |
| Amount | 17 | 100% |

**Characteristics of Respondents According to Types of Cigarettes**

The results of the characteristics of types of cigarettes show that 70.6% of respondents smoke filtered cigarettes. While non-filtered cigarettes are 29.4% of respondents, electronic cigarettes are not used by any respondents. Filters cigarette attractiveness filter ventilation also reduces the irritation

caused by cigarette smoke, making the product more palatable and appealing, and giving smokers the impression that filtered cigarettes are less toxic (18).

Table 5. Distribution of Respondents According to types of cigarettes

| Types of cigarettes | Number | % |
|---|---|---|
| Filter | 12 | 70.6% |
| Non Filter | 5 | 29.4% |
| Electric | 0 | 0% |
| Amount | 17 | 100% |

**Characteristics of Respondents According to Frequency of exposure to cigarette smoke**

Based on the results of the characteristics of frequency of exposure to cigarette smoke exposure to cigarette smoke in smokers and non-smokers. Among smokers, the majority (82.3%) are often passive smokers, 11.8% are rarely exposed, and 5.9% have never been passive smokers. In contrast, among non-smokers, 35.3% are often passive smokers, 47.1% are rarely exposed, and 17.6% have never been passive smokers (Table 6).

Table 6. Distribution of Respondents According *Frequency of exposure to cigarette smoke*

| Exposure to cigarette smoke | Smokers | % | Non-Smokers | % |
|---|---|---|---|---|
| Frequently 3x/Week | 14 | 82.3% | 6 | 35.3% |
| Rarely 1x/Week | 2 | 11.8% | 8 | 47.1% |
| No | 1 | 5.9% | 3 | 17.6% |
| Amount | 17 | 100% | 17 | 100% |

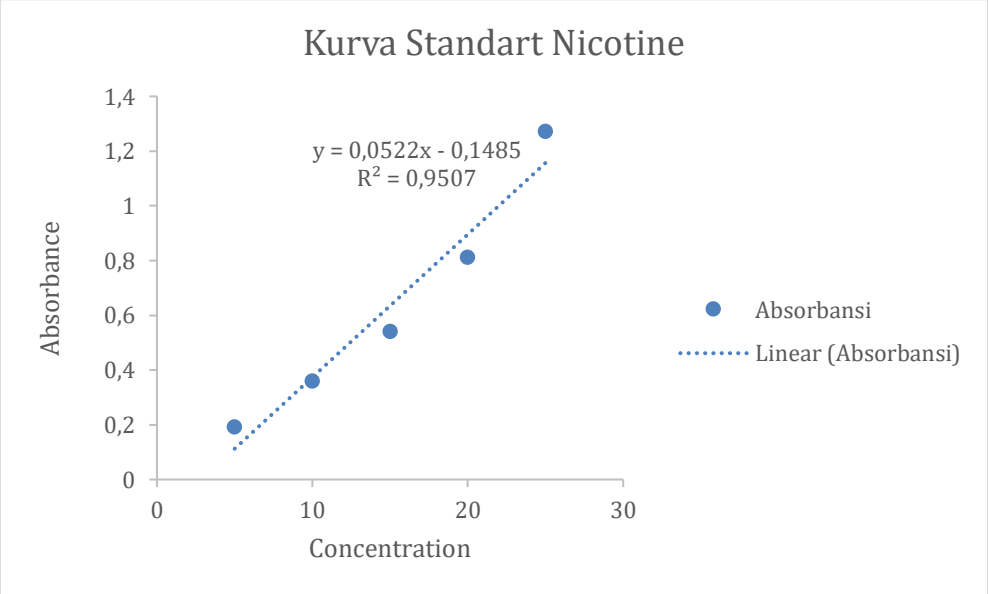


Figure 1. Kurva Standart Nicotine

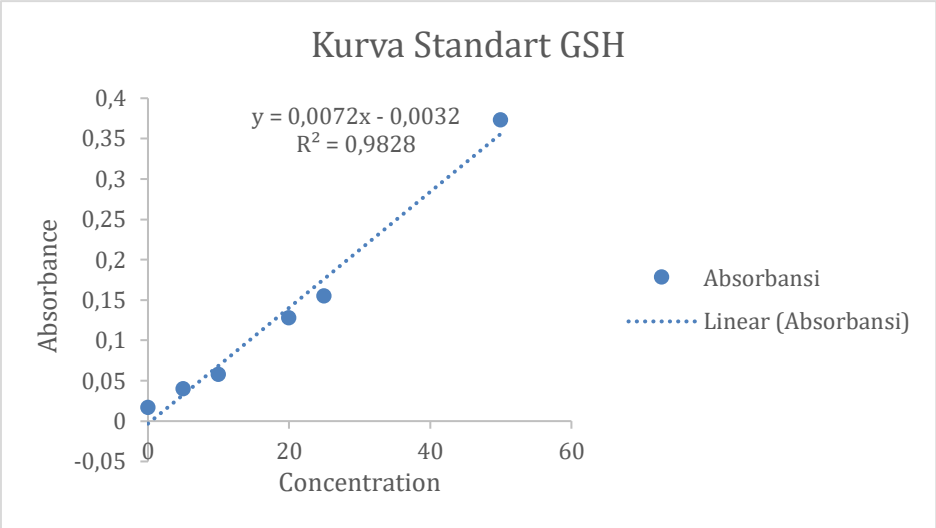


Figure 2. Kurva Standart GSH

**Nicotine and GSH Levels in Smokers and Non-Smokers**

Based on the results of the nicotine level examination, the average nicotine level in smokers was 19.18 µg/ml, while non-smokers were 5.51 µg/ml Table 7. The average GSH level in smokers was 27.00 µg/ml and non-smokers 38.82 µg/ml Table 8.

Table 7. Results of Nicotine and GSH Levels in Smokers

| Respondent Code | Nicotine Level Results (µg/ml) | GSH Level Results (mg/dL) | Normal GSH Value | Normal Nicotine Value |
|---|---|---|---|---|
| P1 | 14.99 | 25.58 | 34-42 mg/dL | 11-52 µg/ml |
| P2 | 15.64 | 24.05 | 34-42 mg/dL | 11-52 µg/ml |
| P3 | 24.65 | 30.44 | 34-42 mg/dL | 11-52 µg/ml |
| P4 | 23.54 | 24.05 | 34-42 mg/dL | 11-52 µg/ml |
| P5 | 23.66 | 18.91 | 34-42 mg/dL | 11-52 µg/ml |
| P6 | 15.53 | 28.36 | 34-42 mg/dL | 11-52 µg/ml |
| P7 | 17.02 | 21.97 | 34-42 mg/dL | 11-52 µg/ml |
| P8 | 25.90 | 23.36 | 34-42 mg/dL | 11-52 µg/ml |
| P9 | 23.15 | 21.41 | 34-42 mg/dL | 11-52 µg/ml |
| P10 | 20.32 | 21 | 34-42 mg/dL | 11-52 µg/ml |
| P11 | 16.86 | 29.88 | 34-42 mg/dL | 11-52 µg/ml |
| P12 | 24.51 | 28.91 | 34-42 mg/dL | 11-52 µg/ml |
| P13 | 15.06 | 29.47 | 34-42 mg/dL | 11-52 µg/ml |
| P14 | 15.97 | 24.75 | 34-42 mg/dL | 11-52 µg/ml |
| P15 | 18.70 | 36.27 | 34-42 mg/dL | 11-52 µg/ml |
| P16 | 15.23 | 46 | 34-42 mg/dL | 11-52 µg/ml |
| P17 | 15.40 | 24.61 | 34-42 mg/dL | 11-52 µg/ml |
| Average | 19.18 | 27.00 | 34-42 mg/dL | 11-52 µg/ml |

Based on the results presented in Table 7, the mean blood nicotine level among smokers was 19.18 µg/mL, which remained within the reported normal reference range (11–52 µg/mL). However, the mean glutathione (GSH) level was 27.00 mg/dL, which was lower than the normal reference range (34–42 mg/dL). These findings suggest that although nicotine exposure in smokers was within the expected range, chronic cigarette smoke exposure may have increased oxidative stress, leading to the

depletion of GSH as an endogenous antioxidant. The reduction in GSH levels indicates a weakened antioxidant defense system, which may increase susceptibility to oxidative damage induced by cigarette smoke.

Tabel 8. *Results of Nicotine and GSH Levels in Non-Smokers*

| Respondent Code | Nicotine Level Results (µg/ml) | GSH Level Results (mg/dL) | Normal GSH Value | Normal Nicotine Value |
|---|---|---|---|---|
| BP1 | 6.97 | 33.5 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP2 | 6.69 | 28.36 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP3 | 4.63 | 38.77 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP4 | 5.26 | 35.16 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP5 | 7.41 | 41.13 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP6 | 5.12 | 30.86 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP7 | 5.83 | 30.86 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP8 | 4.49 | 26.69 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP9 | 4.58 | 48.5 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP10 | 7.44 | 46.27 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP11 | 6.26 | 35.02 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP12 | 4.97 | 51.27 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP13 | 4.53 | 55.16 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP14 | 5.01 | 32.94 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP15 | 4.14 | 47.38 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP16 | 4.46 | 37.66 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| BP17 | 5.83 | 40.44 | 34-42 mg/dL | 0.9-9.07 µg/ml |
| Average | 5.51 | 38.82 | 34-42 mg/dL | 0.9-9.07 µg/ml |

In contrast, non-smokers showed a lower mean nicotine level and a higher mean GSH level than smokers, indicating a better antioxidant status in the absence of direct cigarette smoke exposure. These findings support the role of cigarette smoke as a major source of oxidative stress that contributes to the depletion of endogenous antioxidant reserves.

Tabel 9. Results of the Normality, Homogeneity, and Mann–Whitney Tests for Blood Glutathione (GSH) Levels in Smokers and Non-Smokers

| Normality test | | |
|---|---|---|
| Variable | *p-value* | Information |
| Smokers | 0.014 | Data is not normally distributed |
| Non-Smokers | 0.752 | Data is normally distributed |
| Homogeneity Test | | |
| Variable | p-*value* | Information |
| Smokers | 0.252 | Homogeneous |
| Non-Smokers | 0.252 | Homogeneous |
| Mann Whitney Test | | |
| Variable | *p-value* | Information |
| GSH levels of smokers and nicotine levels of non-smokers | 0.000 | There is a difference |

The Shapiro–Wilk test showed that GSH levels in the smoker group were not normally distributed ($p = 0.014$), whereas the non-smoker group showed a normal distribution ($p = 0.752$). The

homogeneity test indicated equal variances between groups ($p = 0.252$). Therefore, the Mann–Whitney U test was applied, revealing a statistically significant difference in blood GSH levels between smokers and non-smokers ($p < 0.001$). These findings indicate that cigarette smoking is associated with reduced GSH levels, suggesting increased oxidative stress and impaired antioxidant defense in smokers.

## DISCUSSION

This study demonstrated that smokers had significantly higher blood nicotine levels and significantly lower glutathione (GSH) levels than non-smokers. The Mann–Whitney U test showed a statistically significant difference in GSH levels between the two groups ($p < 0.001$). These findings suggest an association between cigarette smoking and reduced antioxidant status, as reflected by lower GSH concentrations in smokers.

The lower GSH levels observed in smokers are biologically plausible because cigarette smoke contains numerous oxidants and free radicals capable of increasing reactive oxygen species (ROS). Excessive ROS production may overwhelm the endogenous antioxidant defense system, leading to increased utilization and depletion of GSH. Since GSH plays a major role in detoxifying ROS and maintaining intracellular redox balance, reduced GSH concentrations may reflect increased oxidative stress associated with cigarette smoke exposure.

The higher nicotine levels observed in smokers are consistent with the pharmacokinetics of nicotine. Following inhalation, nicotine is rapidly absorbed through the pulmonary alveoli into the bloodstream and distributed to various organs. Continuous exposure to cigarette smoke results in sustained nicotine concentrations, while nicotine is subsequently metabolized primarily by the CYP2A6 enzyme into cotinine before being excreted in urine. Individual differences in nicotine metabolism may also contribute to variations in blood nicotine concentrations among smokers.

Our findings are consistent with previous studies reporting that cigarette smoking is associated with increased oxidative stress and decreased antioxidant capacity. Several studies have demonstrated significantly lower GSH concentrations in smokers than in non-smokers, suggesting that chronic exposure to cigarette smoke accelerates antioxidant depletion through persistent free radical generation. Similar findings have also been reported by (20), who observed reduced GSH levels among active smokers, supporting the role of cigarette smoke in disrupting redox homeostasis. However, the magnitude of the differences may vary because of differences in smoking intensity, duration of smoking, age, sex, lifestyle, and laboratory analytical methods.

Although statistically significant differences were observed, the findings should be interpreted with caution because this study employed a cross-sectional design. Therefore, the observed associations do not establish a causal relationship between cigarette smoking and decreased GSH levels. Furthermore, unequal distributions of age and gender between the study groups may have introduced potential confounding bias. Other factors, including dietary intake, alcohol consumption, physical

activity, passive smoking exposure, and underlying health conditions, were also not controlled and may have influenced the measured nicotine and GSH levels.

Despite these limitations, this study provides evidence that smokers tend to have lower GSH levels than non-smokers, indicating impaired antioxidant status associated with cigarette smoke exposure. Future studies with larger sample sizes, age- and gender-matched participants, longitudinal designs, and additional oxidative stress biomarkers are recommended to further clarify the relationship between smoking, nicotine exposure, and antioxidant defense mechanisms.

## CONCLUSIONS AND RECOMMENDATIONS

This study demonstrated that smokers had significantly higher blood nicotine levels and lower glutathione (GSH) levels than non-smokers. These findings indicate that cigarette smoking is associated with reduced antioxidant status and increased oxidative stress. The results highlight the potential value of blood nicotine and GSH as biomarkers for assessing the biological effects of cigarette smoke exposure. This study was limited by the unequal distribution of age and gender between the smoker and non-smoker groups, which may have introduced potential confounding bias. In addition, the cross-sectional design precludes causal inference. Future studies should include larger, age- and gender-matched populations, control for potential confounding factors, and employ longitudinal designs to better clarify the relationship between cigarette smoking, nicotine exposure, and antioxidant status.

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