ORIGINAL RESEARCH

A Comparative Study of Antioxidant Enzyme Activity in Smokers vs. Non-Smokers

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ABSTRACT

Aim: The aim of this study was to evaluate the comparative activity of antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) in smokers versus non-smokers and to assess the associated oxidative stress markers. **Materials and Methods:** A total of 120 participants, including 60 smokers and 60 non-smokers aged 25-50 years, were selected for this study. Venous blood samples were collected to measure the activity of antioxidant enzymes (SOD, CAT, GPx) using spectrophotometric assays. Oxidative stress markers, including malondialdehyde (MDA) and total antioxidant capacity (TAC), were also analyzed. Statistical analysis was performed using independent t-tests. **Results:** Significant reductions in antioxidant enzyme activity were observed in smokers compared to non-smokers (SOD: 1.82 ± 0.29 U/mg vs. 2.34 ± 0.31 U/mg, CAT: 34.5 ± 4.6 U/mg vs. 41.2 ± 5.1 U/mg, GPx: 18.7 ± 2.8 U/mg vs. 22.9 ± 3.0 U/mg, all $p < 0.001$). In contrast, oxidative stress markers were elevated in smokers, with MDA levels significantly higher (5.8 ± 0.9 nmol/mL vs. 3.1 ± 0.7 nmol/mL) and TAC lower (910 ± 102 µmol/L vs. 1150 ± 130 µmol/L, $p < 0.001$). A negative correlation between smoking duration, intensity, and antioxidant enzyme activity was also observed. **Conclusion:** Chronic smoking significantly impairs antioxidant defenses and increases oxidative stress. The results underscore the importance of smoking cessation and lifestyle modifications, including improved diet and physical activity, to mitigate oxidative damage and reduce the risk of smoking-related diseases.

Keywords: Antioxidant Enzymes, Smokers, Oxidative Stress, Superoxide Dismutase, Catalase, Glutathione Peroxidase

INTRODUCTION

The human body is constantly exposed to various internal and external stressors that can lead to the production of reactive oxygen species (ROS). These highly reactive molecules are a natural byproduct of cellular metabolism and play a crucial role in many physiological processes. However, when produced in excess, ROS can lead to oxidative stress, a condition that disrupts the balance between the generation of free radicals and the body's ability to neutralize them through antioxidants. Oxidative stress has been implicated in the pathogenesis of numerous chronic diseases, including cardiovascular disorders, cancer, and respiratory ailments.[1] To combat oxidative stress, the body relies on a complex defense system composed of both enzymatic and non-enzymatic antioxidants. Among the enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are of particular significance. These enzymes act synergistically to scavenge and neutralize harmful free radicals, thus protecting cells and tissues from oxidative damage. The efficiency and activity of these enzymes are critical indicators of the body's ability to manage oxidative stress and maintain cellular homeostasis.[2] Cigarette smoking is one of the most prevalent and preventable causes of morbidity and mortality worldwide. It introduces a substantial amount of harmful substances into the body, including nicotine, carbon monoxide, and more than 4,000 other chemical compounds. Among these, many are known to promote oxidative stress either directly by generating free radicals or indirectly by depleting the body's natural antioxidant reserves. The lungs, being the primary site of exposure, are especially vulnerable to oxidative damage, but the systemic effects of smoking also impact various organs and tissues. Over time, this oxidative burden contributes significantly to the

development and progression of smoking-related diseases.[3] Given the known oxidative potential of cigarette smoke, it is reasonable to hypothesize that smoking can alter the body's antioxidant defense mechanisms, particularly the activity of enzymatic antioxidants such as SOD, CAT, and GPx. Understanding these changes is crucial, not only for elucidating the biochemical effects of smoking but also for developing potential interventions that could mitigate its harmful consequences. Comparing the antioxidant enzyme activity between smokers and non-smokers provides valuable insight into how lifestyle choices influence oxidative stress and the body's defensive responses.[4]In smokers, continuous exposure to the oxidative compounds in cigarette smoke may lead to either upregulation or suppression of antioxidant enzymes. Some studies suggest that chronic exposure initially stimulates an adaptive increase in enzyme activity as the body attempts to counteract the heightened oxidative stress. However, over time, the persistent oxidative insult may overwhelm this compensatory mechanism, leading to a depletion or dysfunction of antioxidant enzymes. On the other hand, non-smokers, who are generally exposed to lower levels of exogenous oxidative stress, may maintain more stable and efficient antioxidant enzyme activity, reflective of a balanced redox state.[5] Moreover, individual variability in antioxidant enzyme activity can be influenced by several factors, including age, gender, dietary habits, genetic predisposition, and environmental exposures. However, smoking stands out as a modifiable risk factor that has a direct and profound impact on oxidative stress levels. Therefore, isolating its effect by comparing groups of smokers and non-smokers under controlled conditions can yield meaningful data about the biochemical shifts associated with tobacco use.[6] This comparative study aims to investigate the differences in antioxidant enzyme activity, specifically focusing on SOD, CAT, and GPx, between smokers and non-smokers. By examining the activity levels of these key enzymes in both groups, this research seeks to identify patterns that reflect the influence of smoking on the body's oxidative defense mechanisms. Such data could not only reinforce the understanding of smoking-induced oxidative stress but also aid in the development of targeted antioxidant therapies or public health strategies aimed at reducing the burden of smoking-related diseases.[7] Additionally, this study may contribute to the growing field of preventive medicine, where biomarker profiling can help assess individual risk and guide personalized health recommendations. If significant differences in enzyme activity are observed, they could potentially serve as early indicators of oxidative stress and tissue damage in smokers, even before clinical symptoms manifest. This opens avenues for early interventions and monitoring strategies that focus on maintaining or restoring antioxidant balance in high-risk populations.

MATERIALS AND METHODS

This comparative study was conducted on a total of 120 individuals, comprising 60 smokers and 60 non-smokers, to evaluate the activity of antioxidant enzymes between the two groups. Participants were selected from outpatient clinics and through community-based health camps, with age ranging from 25 to 50 years. Individuals with a history of chronic diseases such as diabetes, cardiovascular disorders, or those on antioxidant supplements were excluded to minimize confounding variables. Informed consent was obtained from all participants, and ethical approval was granted by the institutional review board. Smokers were defined as individuals who had been smoking at least five cigarettes per day for the past five years, while non-smokers had no history of tobacco use in any form.

Venous blood samples (5 mL) were collected under aseptic conditions from all participants and immediately processed. Erythrocytes were separated, and the levels of antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—were measured using spectrophotometric assays following standard biochemical protocols. Protein content in samples was estimated using the Lowry method to normalize enzyme activity. All assays were conducted in triplicate to ensure reproducibility of results. Statistical analysis was performed using SPSS software version 21.0. Independent t-tests were used to compare the mean enzyme activities between smokers and non-smokers, with a p-value of less than 0.05 considered statistically significant.

RESULTS

Demographic and Lifestyle Characteristics

The study included 120 participants, equally divided into 60 smokers and 60 non-smokers. The mean age of smokers was 37.6 ± 6.2 years, which was not significantly different from non-smokers, whose mean age was 36.8 ± 5.9 years ($p = 0.47$). Gender distribution was also comparable, with the smoker group comprising 48 males and 12 females, and the non-smoker group comprising 45 males and 15 females ($p = 0.52$). The mean Body Mass Index (BMI) for smokers was 24.3 ± 2.1 kg/m², slightly higher than non-smokers (23.9 ± 2.3 kg/m²), but this difference was not statistically significant ($p = 0.36$).

Lifestyle-related parameters, however, showed significant differences between the groups. Alcohol consumption was more prevalent among smokers (65%) compared to non-smokers (36.7%), which was statistically significant ($p < 0.01$). Physical activity was reported regularly by only 14 smokers compared to 32 non-smokers, indicating a sedentary trend among smokers ($p < 0.001$). Dietary habits were also poorer in smokers, with only 18 reporting a balanced diet, as opposed to 39 non-smokers ($p < 0.001$). Biochemically, total protein levels were significantly lower in smokers (6.5 ± 0.7 g/dL) compared to non-

smokers (7.1 ± 0.6 g/dL) ($p < 0.001$), reflecting potential nutritional or oxidative stress-related imbalances.

Antioxidant Enzyme Activity and Oxidative Stress Markers

There was a clear and significant reduction in the activity of antioxidant enzymes in smokers compared to non-smokers. Superoxide dismutase (SOD) levels were significantly lower in smokers (1.82 ± 0.29 U/mg protein) than in non-smokers (2.34 ± 0.31 U/mg) ($p < 0.001$). Similarly, catalase (CAT) activity was decreased in smokers (34.5 ± 4.6 U/mg) compared to non-smokers (41.2 ± 5.1 U/mg) ($p < 0.001$). Glutathione peroxidase (GPx) levels also followed this trend, with smokers exhibiting 18.7 ± 2.8 U/mg as opposed to 22.9 ± 3.0 U/mg in non-smokers ($p < 0.001$).

In contrast, oxidative stress markers were elevated in smokers. The level of malondialdehyde (MDA), a marker of lipid peroxidation, was significantly higher in smokers (5.8 ± 0.9 nmol/mL) than in non-smokers (3.1 ± 0.7 nmol/mL) ($p < 0.001$). Additionally, total antioxidant capacity (TAC) was markedly reduced in smokers (910 ± 102 µmol/L) in comparison to non-smokers (1150 ± 130 µmol/L) ($p < 0.001$), indicating an overall depletion of the antioxidant defense system due to smoking.

Smoking Patterns and Intensity

Among the smokers, the duration of smoking varied, with 36.7% smoking for 5–10 years, 30.0% for 11–15 years, 20.0% for 16–20 years, and 13.3% for more than 20 years. In terms of intensity, 43.3% smoked 5–10 cigarettes per day, 35.0% smoked 11–20, and 21.7% smoked more than 20 per day. The average pack-years among smokers was calculated to be 13.8 ± 4.1, reflecting cumulative exposure to tobacco. Notably, 19 smokers also reported passive smoking exposure at home, compared to only 8 among non-smokers, which could further exacerbate oxidative stress in this group.

Correlation Between Smoking Variables and Antioxidant Enzymes

A significant negative correlation was found between smoking-related variables and antioxidant enzyme activity. Smoking duration showed a strong inverse correlation with SOD ($r = -0.61$), CAT ($r = -0.58$), and GPx ($r = -0.64$), and a positive correlation with MDA levels ($r = +0.69$), all statistically significant at $p < 0.01$. Similarly, the number of cigarettes smoked per day negatively correlated with antioxidant enzyme levels and positively with MDA, indicating dose-dependent oxidative stress. Pack-years also demonstrated a strong negative correlation with antioxidant enzyme activity (SOD $r = -0.64$; CAT $r = -0.60$; GPx $r = -0.65$) and a strong positive correlation with MDA ($r = +0.72$), confirming the cumulative effect of smoking.

Alcohol consumption negatively impacted antioxidant enzyme levels and positively affected MDA levels, though to a slightly lesser extent ($p < 0.05$). Interestingly, regular physical activity was positively associated with higher antioxidant enzyme activity (SOD $r = +0.39$; CAT $r = +0.41$; GPx $r = +0.37$) and lower MDA ($r = -0.44$), suggesting a protective role of exercise against oxidative stress in both groups.

Percentage Differences in Biomarkers

The comparative percentage differences in biomarker levels between smokers and non-smokers highlighted the adverse impact of smoking. Superoxide dismutase activity was reduced by 22.2% in smokers, catalase by 16.3%, and glutathione peroxidase by 18.3%. In contrast, MDA levels were elevated by 87.1%, showing a near doubling of lipid peroxidation in smokers. Total antioxidant capacity was reduced by 20.9% in the smoking group. These differences collectively underscore the significant oxidative burden imposed by smoking and the concurrent suppression of the body's natural antioxidant defense mechanisms.

Table 1: Demographic and Lifestyle Characteristics of Study Participants

Parameter	Smokers (n = 60)	Non-Smokers (n = 60)	p-value
Age (mean ± SD, years)	37.6 ± 6.2	36.8 ± 5.9	0.47
Gender (Male/Female)	48 / 12	45 / 15	0.52
BMI (kg/m²)	24.3 ± 2.1	23.9 ± 2.3	0.36
Alcohol Consumption (Yes/No)	39 / 21	22 / 38	< 0.01
Physical Activity (Regular/None)	14 / 46	32 / 28	< 0.001
Dietary Pattern (Balanced/Poor)	18 / 42	39 / 21	< 0.001
Total Protein (g/dL)	6.5 ± 0.7	7.1 ± 0.6	< 0.001

Table 2: Mean Antioxidant Enzyme Activity and Oxidative Stress Markers

Parameter	Smokers (mean ± SD)	Non-Smokers (mean ± SD)	p-value
Superoxide Dismutase (U/mg)	1.82 ± 0.29	2.34 ± 0.31	< 0.001
Catalase (U/mg)	34.5 ± 4.6	41.2 ± 5.1	< 0.001
Glutathione Peroxidase (U/mg)	18.7 ± 2.8	22.9 ± 3.0	< 0.001
Malondialdehyde (MDA, nmol/mL)	5.8 ± 0.9	3.1 ± 0.7	< 0.001

Total Antioxidant Capacity (TAC, µmol/L)	910 ± 102	1150 ± 130	< 0.001

Table 3: Distribution of Smokers by Smoking Patterns and Intensity

Parameter	Number (n = 60)	Percentage (%)
Smoking Duration (years)		
– 5–10	22	36.7
– 11–15	18	30.0
– 16–20	12	20.0
– > 20	8	13.3
Cigarettes per Day		
– 5–10	26	43.3
– 11–20	21	35.0
– > 20	13	21.7
Pack-Years (mean ± SD)	13.8 ± 4.1	–
Passive Smoking at Home (Yes/No)	19 / 41	8 / 52

Table 4: Correlation Between Smoking-Related Variables and Antioxidant Enzymes

Parameter	SOD (r)	CAT (r)	GPx (r)	MDA (r)	p-value
Smoking Duration (years)	-0.61	-0.58	-0.64	+0.69	< 0.01
Cigarettes per Day	-0.56	-0.53	-0.59	+0.66	< 0.01
Pack-Years	-0.64	-0.60	-0.65	+0.72	< 0.01
Alcohol Consumption	-0.48	-0.42	-0.47	+0.51	< 0.05
Physical Activity	+0.39	+0.41	+0.37	-0.44	< 0.05

Table 5: Percentage Differences in Oxidative Stress Biomarkers Between Groups

Biomarker	Non-Smokers (Mean)	Smokers (Mean)	% Difference	Direction
Superoxide Dismutase (U/mg)	2.34	1.82	-22.2%	↓
Catalase (U/mg)	41.2	34.5	-16.3%	↓
Glutathione Peroxidase (U/mg)	22.9	18.7	-18.3%	↓
Malondialdehyde (nmol/mL)	3.1	5.8	+87.1%	↑
Total Antioxidant Capacity (µmol/L)	1150	910	-20.9%	↓

DISCUSSION

The demographic characteristics in this study showed no statistically significant difference in age, gender, or BMI between smokers and non-smokers, ensuring a comparable baseline. However, lifestyle-related differences such as higher alcohol consumption and lower physical activity among smokers were significant and may contribute to oxidative stress. These findings are consistent with those of **Aydın et al. (2013)**, who reported that smokers were more likely to engage in unhealthy behaviors, including excessive alcohol intake and poor dietary patterns, which compounded the oxidative burden associated with tobacco use.[8]

The significant reduction in antioxidant enzyme activity observed in smokers in this study—SOD (1.82 ± 0.29 U/mg vs. 2.34 ± 0.31 U/mg), CAT (34.5 ± 4.6 U/mg vs. 41.2 ± 5.1 U/mg), and GPx (18.7 ± 2.8 U/mg vs. 22.9 ± 3.0 U/mg)—demonstrates the suppressive effect of tobacco-related free radicals on enzymatic defense mechanisms. These results closely mirror the findings of **Rahman et al. (2006)**, who observed a similar trend in smokers, with a notable 20–25% reduction in SOD and CAT activity, attributing the decline to chronic exposure to reactive oxygen species (ROS) in cigarette smoke.[9]

Oxidative stress markers further confirmed this trend, with smokers exhibiting significantly higher MDA levels (5.8 ± 0.9 nmol/mL) and lower TAC (910 ± 102 µmol/L) compared to non-smokers (3.1 ± 0.7 nmol/mL and 1150 ± 130 µmol/L, respectively). Elevated MDA indicates increased lipid peroxidation, a key indicator of oxidative damage. This aligns with findings by **Altintás et al. (2010)**, who reported MDA levels nearly double in smokers compared to non-smokers, highlighting the vulnerability of lipids to free radical attack due to smoking.[10]

The relationship between smoking intensity and antioxidant status in this study also revealed a dose-dependent impact. Smokers with greater duration and quantity of smoking showed more pronounced reductions in antioxidant enzyme levels and increased MDA concentrations. These results are comparable to those reported by **Armutcu et al. (2004)**, who demonstrated that antioxidant defenses declined progressively with increased smoking pack-years and that MDA levels rose correspondingly, suggesting a cumulative oxidative insult over time.[11]

Correlation analysis in the present study established strong negative relationships between smoking-related variables and antioxidant enzyme levels (e.g., pack-years vs. SOD, r = -0.64), while MDA levels

correlated positively with smoking duration and intensity. **Jain et al. (2000)** also reported similar correlations, particularly between pack-years and MDA (r = +0.71), reinforcing the concept that chronic smoking exacerbates oxidative damage through both direct and indirect pathways.[12]

Finally, the percentage differences observed—22.2% reduction in SOD, 16.3% in CAT, 18.3% in GPx, and an 87.1% increase in MDA—highlight the severe biochemical impact of smoking. These values are comparable to those reported by **Dietrich et al. (2003)**, who documented a 15–30% reduction in enzymatic antioxidants and more than a 70% elevation in lipid peroxidation markers in chronic smokers. Such findings strongly support the hypothesis that smoking overwhelms the body's endogenous antioxidant systems, resulting in systemic oxidative stress.[13]

CONCLUSION

This study demonstrated that chronic smoking significantly impairs antioxidant defense mechanisms and increases oxidative stress, as evidenced by reduced levels of SOD, CAT, and GPx, along with elevated MDA and decreased TAC in smokers compared to non-smokers. The severity of oxidative imbalance was found to correlate with the duration and intensity of smoking. Lifestyle factors such as poor diet, alcohol consumption, and physical inactivity further compounded oxidative damage in smokers. These findings emphasize the need for smoking cessation and lifestyle modification to restore antioxidant balance and reduce the risk of oxidative stress-related diseases.

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