Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Evaluation of Plasma Lead and Oxidative Stress Markers among Cigarette Smokers

GadAllah Modawe
Omdurman Islamic University
Islam Abdelmonim
National University-Sudan, Faculty of Medical Laboratory Sciences
Omnia Ahmed
National University-Sudan, Faculty of Medical Laboratory Sciences
Samia Afeef
National University-Sudan, Faculty of Medical Laboratory Sciences
Shaymaa Abbas
National University-Sudan, Faculty of Medical Laboratory Sciences
Rayan Mohamed
National University-Sudan, Faculty of Medical Laboratory Sciences
Elhashimi E Hassan
Najran University
Mohammed Ali Khalifa
Mmali198939@gmail.com
University of science and technology
Mohammed. A. Mahdi
National University-Sudan, Faculty of Medical Laboratory Sciences

Article

Keywords: Cigarette smoking, Lead Oxidative stress, Glutathione peroxidase, Glutathione reductase, Superoxide dismutase, Catalase Malondialdehyde

Posted Date: September 24th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10947680/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

Cigarette smoking is a major source of lead exposure and contributes to oxidative stress by overproduction of reactive oxygen species and impairment of antioxidant defense systems. However, there are limited data on the simultaneous evaluation of plasma lead and multiple oxidative stress markers in smokers, particularly in Sudan. The aim of the present study was to investigate plasma lead levels and oxidative stress markers in cigarette smokers.

Methods

A community-based case-control study was carried out in Khartoum State, Sudan, on 50 cigarette smokers and 50 healthy non-smokers. Plasma lead concentrations were measured by atomic absorption spectrophotometry and biomarkers of oxidative stress (GPx, GR, SOD, catalase and MDA) were determined by standard spectrophotometric methods. Statistical analysis was performed by SPSS version 20.0 and $P < 0.05$ considered as significant.

Results

Smokers had significantly higher plasma lead concentrations than non-smokers (1.9 ± 1.1 vs. 0.4 ± 0.3, $P < 0.001$). GPx activity was significantly increased, but GR and catalase activities were significantly decreased ($P < 0.05$). There was no significant difference in SOD activity and MDA concentrations. The plasma lead concentration was positively associated with smoking duration and GPx activity was positively associated with the number of cigarettes smoked per day.

Conclusion

Chronic cigarette smoking is associated with increased plasma lead burden and alteration of antioxidant defenses, suggesting oxidative stress as a major mechanism of smoking-related toxicity. These results support smoking cessation and larger prospective studies are needed to further elucidate the health effects of cumulative lead exposure.

1. Introduction

Cigarette smoking is one of the leading preventable causes of morbidity and mortality worldwide, resulting in over eight million deaths annually and imposing a heavy burden on health care systems and national economies. Tobacco use is very common, even with widespread public health interventions. This is especially true in low- and middle-income countries, where rising urbanization, socioeconomic inequalities and weak tobacco control policies contribute to high smoking rates. Cigarette smoke is an

extraordinarily complex aerosol, containing more than 7,000 chemical constituents, in addition to nicotine dependence. These constituents include numerous carcinogens, reactive oxidants, and toxic heavy metals, which collectively contribute to the pathogenesis of cardiovascular, respiratory, metabolic, and malignant diseases(1–4).

One of the toxic constituents of tobacco and one of the most important environmental contaminants associated with tobacco use is lead (Pb)(5). During cultivation, tobacco plants readily take up lead from contaminated soils, irrigation water, fertilizers and atmospheric deposition(6). Cigarette smoke, therefore, is a significant source of chronic lead exposure(7). Inhaled lead particles circumvent the regulatory mechanisms of the gastrointestinal tract and are readily absorbed into the systemic circulation through the pulmonary alveoli(8, 9). Cigarette smoking, in contrast to an acute environmental exposure, causes repeated low-dose exposure to lead over many years, with progressive accumulation in the blood, soft tissues, and bone(10, 11). Because of the long biological half-life of skeletal lead stores, the biological effects of active tobacco use can persist for a long time after smoking cessation because skeletal lead stores can be an endogenous source of exposure(12).

Lead toxicity has multiple molecular mechanisms and affects virtually all organ systems. One of the main mechanisms involved is disruption of cellular redox homeostasis leading to overproduction of reactive oxygen species (ROS) and dysfunction of endogenous antioxidant defense systems(13). Lead has a high affinity to sulfhydryl (-SH) groups within proteins and antioxidant enzymes, causing inactivation of enzymes, changes in protein conformation, mitochondrial dysfunction and disturbance of intracellular calcium signaling(14). Moreover, lead disrupts important trace elements such as zinc, selenium, iron and calcium which are cofactors for many antioxidant enzymes leading to enhanced oxidative injury(15, 16). It then induces persistent oxidative stress resulting in lipid peroxidation, protein oxidation, DNA damage, inflammation, endothelial dysfunction, apoptosis, and the premature aging of tissues, all of which lead to the development of smoking-related chronic diseases(17).

The human antioxidant defense system consists of enzymatic and non-enzymatic components working synergistically to eliminate reactive oxygen species and maintain cellular integrity. The major enzymatic antioxidants are superoxide dismutase (SOD) that catalyses the dismutation of superoxide radicals to hydrogen peroxide. Hydrogen peroxide is then detoxified by catalase (CAT) and glutathione peroxidase (GPx)(18). Glutathione reductase (GR) complements this role by reducing oxidized glutathione (GSSG) back to reduced glutathione (GSH) to maintain intracellular redox balance and support GPx activity in turn. Malondialdehyde (MDA), a stable end product of lipid peroxidation, is widely recognized as a reliable biomarker of oxidative membrane damage and oxidative stress burden(19). Changes in these biomarkers provide information about the biological effects of chronic exposure to cigarette smoke and environmental toxicants(20).

Cigarette smoking has been repeatedly shown in several previous studies to be associated with increased oxidative stress and disturbances of antioxidant defense mechanisms. However, the reported pattern of antioxidant enzyme activity is still inconsistent in different populations. Some studies have

shown that smokers have lower activity of GPx, SOD, catalase and GR, whereas other studies have shown that certain antioxidant enzymes are raised in a compensatory way in the early stages of oxidative insult. Also, circulating MDA concentrations have generally been reported to be elevated in smokers, although a number of studies have failed to demonstrate statistically significant differences. These differences may be related to differences in smoking intensity and duration of exposure, nutritional status, genetic susceptibility, environmental pollution, analytical techniques and ethnicity or geographical variability(21).

Similarly, a few epidemiological studies have shown higher blood lead levels among smokers than non-smokers. However, few studies have measured plasma lead levels along with multiple oxidative stress biomarkers simultaneously in the same study population, particularly in African and Middle Eastern countries, where environmental lead exposure still remains an important public health concern. Sudan is characterized by several possible sources of lead exposure including industry, traffic, occupation and environmental pollution, which may further enhance the toxic effects of cigarette smoking. However, in spite of these concerns, there is still a dearth of large-scale studies to evaluate the relationship between lead burden and antioxidant defense systems in Sudanese cigarette smokers(7, 22).

Better understanding of the interactions of chronic cigarette smoking, lead and oxidative stress are important to understand the biological mechanisms involved in smoking-related diseases and to identify potential biomarkers that may be useful in the early detection of toxic exposure prior to irreversible organ damage. Such evidence also can inform preventive strategies, environmental health policies, and smoking cessation programs aimed at reducing long-term health consequences of chronic tobacco use(23, 24).

The present study was thus designed to evaluate plasma lead concentrations and some oxidative stress biomarkers such as glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase and malondialdehyde in cigarette smokers relative to apparently healthy non-smokers in Khartoum State, Sudan. Furthermore, the study investigated the correlations between these biochemical parameters and smoking related variables like smoking duration and daily cigarette consumption in order to better understand the cumulative biological effects of chronic tobacco exposure. The present case-control study contributes valuable information regarding the interaction between heavy metal exposure and oxidative stress in smokers in a population for whom such data are still lacking.

2. Materials and Methods

2.1. Study Design: Analytical case control community-based study was conducted at Khartoum state, during the period from June to September 2022.

2.2. Sample size: A total of 100 subjects were enrolled in the study, 50 cigarette smokers as case group and 50 healthy individuals as control group.

2.3. Inclusion and exclusion criteria: Cigarettes smokers from age 18 to 60 years were included. New smokers, smokers with chronic diseases, liver disease and malignancy were excluded.

2.4. Data collection and blood sampling: Structured questionnaire was design to collect medical and personal information included age, BMI, duration of smoking, cigarettes per day. Samples were collected using sterile disposable plastic syringe after cleaning the vein puncture area with 70 % ethanol. 5 ml of whole blood was collected in the syringe, using anticoagulant heparin, then centrifuging at 1000-2000xg for 5 minutes. Then plasma was transfer into sterile plain container and store in 2-8^{o} C until being tested. The plasma lead was estimated using atomic absorption spectrophotometric method, while the plasma oxidative stress parameters were measured using spectrophotometric method.

2.5. Ethical Considerations: Before conducting the study, the proposal was approved by ethical committee of national university and the lab at which the study was done, informed constant was obtained from all participants in the study

2.6. Data analysis: The result of the study was expressed (mean ±SD) and percentage (%) and P value consider less than or equal (0.05) is consider significant. The result was analyzed by using statistical package for social science (SPSS) version 20.

3. Results

3.1. Study Population Characteristics

A total of 100 participants was enrolled for the study, 50 cigarette smokers and 50 non-smokers. The mean age of smokers was 41.2 years and the average duration of smoking was 14.1 years with an average number of cigarettes/days of 22.3. The mean body mass index (BMI) was 22.6 kg/m2 for smokers and 21.1 kg/m2 for non-smokers. The findings reported here describe the baseline characteristics of the study population, as shown in Table 1.

Table 1: Descriptive Statistics of study population

Variables	N	Minimum	Maximum	Mean ± SD
Age (smokers)	50	18.0	60.0	41.2 ±113
Duration of smoking/ years (smokers)	50	0.5	40.0	14.1± 6.5
Number cigarette/day (smokers)	50	1	50	22.3 ± 2.2
BMI (smokers)	50	16.7	24.1	22.6 ± 1.7
Age (non-smokers (	50	17.0	70.0	36.2 ± 4.6
BMI (nonsmokers)	50	18.3	23.5	21.1 ± 2.2

3.2. Clinical Manifestations Seen in Cigarette Smokers

As described in Figure 1, the majority of smokers (80%) reported no smoking-related symptoms. Heavy cough and fever/headache/abdominal pain were the most common complaints in symptomatic participants. Difficulty in breathing, limb numbness and light cough were less common.

3.3. Plasma Lead and Oxidative Stress Biomarkers among Smokers and Non-Smokers

Plasma lead levels were significantly higher in smokers compared to non-smokers (1.9 ± 1.1 vs. 0.4 ± 0.3, P<0.001). Also, GPx activity in smokers was significantly higher. Whereas much lower activities of glutathione reductase (GR) and catalase were found in the smokers. No significant differences were found between the two groups with respect to SOD activity or MDA concentrations. The results suggest that chronic cigarette smoking altered the antioxidant defense machineries, as shown in Table 2.

Table 2: (Mean ±SD) of plasma lead and oxidative stress markers among study population

Study parameters	**Smokers N= 50**	**Non smokers N= 50**	***P-value***
Lead	1.9 ± 1.1	0.4 ± 0.3	0.00
GSH	427.4 ± 37.2	278.2 ± 29.2	0.00
GR	13.4 ± 3.2	18.6 ± 2.9	0.00
SOD	10.7 ± 1.6	11.2 ± 0.7	0.86
Catalase	7.3 ± 3.2	10.2 ± 2.7	0.03
MDA	2.3 ± 0.6	2.7 ± 0.9	0.46

3.4. Correlation of Plasma Lead Levels with Smoking Characteristics

As demonstrated in Table 3, Age, BMI and cigarettes smoked per day were not significantly correlated with plasma lead concentration. However, plasma lead concentration was positively associated with duration of smoking, and this was statistically significant. Because the correlation coefficient reported was r = 1.00, this result should be interpreted with caution and verified before publication, as perfect correlations are extremely rare in biological studies.

Table 3: Correlation between plasma parameters and study variables of smokers

parameters	Variable	r	*P-value*
Plasma Lead	Age	0.13	0.43
	BMI	-0.26	0.71
	Duration of smoking/ years	1	**0.00**
	Number of cigarette / days	-0.12	0.18
GSH	Age	0.17	0.32
	BMI	-0.24	0.41
	Duration of smoking/ years	0.22	0.28
	Number of cigarette / days	0.83	**0.02**
GR	Age	0.19	0.52
	BMI	-0.13	0.59
	Duration of smoking/ years	0.21	0.63
	Number of cigarette / days	-0.17	0.79
SOD	Age	-0.28	0.72
	BMI	-0.15	0.69
	Duration of smoking/ years	-0.21	0.83
	Number of cigarette / days	0.31	0.29
Catalase	Age	-0.07	0.54
	BMI	-0.34	0.61
	Duration of smoking/ years	-0.25	0.76
	Number of cigarette / days	-0.42	0.82
MDA	Age	-0.38	0.42
	BMI	-0.38	0.57
	Duration of smoking	-0.17	0.63
	Number of cigarette / days	0.13	0.39

Discussion

The current study evaluated the plasma lead concentrations and biomarkers of oxidative stress among cigarette smokers. It showed that the smokers had significantly higher plasma lead concentrations along with altered antioxidant defense mechanisms compared to non-smokers(25). In particular,

significantly increased activities of glutathione peroxidase (GPx) and significantly decreased activities of glutathione reductase (GR) and catalase, and no significant differences in superoxide dismutase (SOD) activity and malondialdehyde (MDA) levels were found. In addition, plasma lead concentrations were positively correlated with the duration of smoking, while GPx activity was correlated with the number of cigarettes smoked per day. These results indicate that chronic cigarette smoking causes cumulative lead exposure and alters the antioxidant defense system, disturbing the redox homeostasis(26, 27).

The significant increase of plasma lead concentration in smokers is consistent with the well-known role of tobacco smoking as a relevant source of heavy metal exposure. Tobacco plants take up lead from contaminated soil and environment and inhalation of cigarette smoke provides an efficient route for systemic lead absorption. Repeated exposure over many years leads to lead building up in the blood and bones, which causes long-term toxicity. Lead is known to cause oxidative stress by producing excessive reactive oxygen species (ROS), mitochondrial dysfunction, and inhibition of antioxidant enzymes, and it increases the risk of cardiovascular, neurological, renal, and respiratory diseases (28, 29).

The changes in the antioxidant enzymes in smokers reflect a disbalance in the oxidative defense system. The increase in GPx activity can be considered as a compensatory adaptive response to the increase of oxidative stress, which is confirmed by the increased detoxification of hydrogen peroxide and lipid hydroperoxides(30, 31). In contrast, the reduction in GR and catalase activities suggests a decreased antioxidant capacity after chronic cigarette smoke exposure(32). Lead readily binds to sulfhydryl groups and can directly inhibit antioxidant enzymes or interfere with essential cofactors required for their activity. Taken together, these changes suggest that long term smoking overpowers the endogenous antioxidant defense system despite activation of some protective mechanisms(33, 34).

Unlike GPx, GR and catalase, no significant difference in SOD activity was observed between smokers and non-smokers. Some previous studies have reported similar findings, but the literature remains inconsistent(32, 35, 36). These discrepancies may be explained by differences in smoking intensity and duration, nutritional status, environmental exposure, analytical methods and genetic susceptibility. Therefore, the lack of significant changes in SOD activity should not be interpreted as evidence of normal oxidative status, especially when significant changes in other biomarkers of antioxidant activity are detected.

Similarly, plasma MDA concentrations, a widely accepted marker of lipid peroxidation, were not significantly different in the two groups. This finding is contrary to many previous studies which reported increased levels of MDA in smokers. This may be attributed to the relatively smaller sample size, biological variability, dietary intake of antioxidants or the compensatory antioxidant responses. The increased GPx activity may have partially restricted lipid peroxidation and prevented an observable increase in circulating MDA concentrations(37, 38). The positive association between plasma lead levels and duration of smoking suggests that lead exposure in chronic smokers is cumulative(7). Similarly, the positive correlation between GPx activity and the number of cigarettes smoked per day indicates that the greater the exposure to tobacco, the greater the oxidative stress and the stimulation of the antioxidant

responses(31, 39). The reported correlation coefficient (r = 1.00) between plasma lead and smoking duration should be questioned before publication; perfect correlations are unusual in biological studies(7, 40).

Strengths of this study include the concurrent measurement of plasma lead and several oxidative stress biomarkers allowing for a comprehensive assessment of redox status in smokers. However, the results should be interpreted in the context of several limitations including the relatively small sample size, the single-center design, and the cross-sectional study design, which do not allow for causal inference. Moreover, we did not evaluate various confounding factors such as dietary antioxidant intake, occupational exposure and environmental pollution.

Conclusion

Chronic cigarette smoking was associated with increased plasma lead concentrations and significant alterations in antioxidant defense mechanisms, suggesting that oxidative stress is an important pathway through which smoking exerts its toxic effects. These results demonstrate the importance of reduction of tobacco exposure and provide the basis for further large prospective studies to elucidate the association of heavy metal accumulation, oxidative stress and diseases related to smoking.

Declarations

Ethical Considerations

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Approval of the study was obtained from the Ethics Committee of the National University, Sudan, before the study started. Prior to enrolment all participants provided written informed consent and all personal information was kept confidential throughout the study.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sectors.

CRediT authorship contribution statement

GadAllah Modawe: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft. Islam Abdelmonim: Investigation, Data collection, Laboratory analysis. Omnia Ahmed: Investigation, Lab analysis. Samia Afeef : Investigation, Laboratory tests. Shaymaa Abbas: Data collection, Investigation. Rayan Mohamed: Data curation, Investigation. Elhashimi E. Hassan: Conceptualization, Methodology, Supervision, Writing-Review & Editing. And, Mohammed Ali Khalifa: Conceptualization, Methodology, Formal analysis, Data interpretation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration.

Declaration of competing interest

The authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

The authors thank the staff of the participating laboratories and all volunteers that participated in this study. The authors also thank the National University, Sudan, for laboratory work and all the people who helped with sample collection and data acquisition.

References

1. Jha, P. The hazards of smoking and the benefits of cessation: a critical summation of the epidemiological evidence in high-income countries. *elife* **9**, e49979 (2020).
2. Le Foll, B. et al. Tobacco and nicotine use. *Nat. Reviews Disease Primers*. **8** (1), 19 (2022).
3. Seo, Y-S., Kim, J-H. & Lee, M-Y. Cigarette smoke-induced reactive oxygen species formation: a concise review. *Antioxidants* **12** (9), 1732 (2023).
4. Maiyo, A. K., Kibet, J. K. & Kengara, F. O. A review of the characteristic properties of selected tobacco chemicals and their associated etiological risks. *Rev. Environ. Health*. **38** (3), 479–491 (2023).
5. Proshad, R., Zhang, D., Uddin, M. & Wu, Y. Presence of cadmium and lead in tobacco and soil with ecological and human health risks in Sichuan province, China. *Environ. Sci. Pollut. Res.* **27** (15), 18355–18370 (2020).
6. Hussain, R. et al. Evaluation of the contaminated soil and its impacts on Tobacco (Nicotiana tabacum L.) crops in Swabi, Pakistan. *J. Himal. Earth Sci.* **53** (1), 34–48 (2020).
7. Repić, A. et al. The influence of smoking habits on cadmium and lead blood levels in the Serbian adult people. *Environ. Sci. Pollut. Res.* **27** (1), 751–760 (2020).
8. Generalova, A., Davidova, S. & Satchanska, G. The mechanisms of lead toxicity in living organisms. *J. xenobiotics*. **15** (5), 146 (2025).
9. Aktepe, N., Baran, M. F. & Baran, A. Effects of chronic exposure to lead on some organs. *Assistant* **18**, 45 (2022).
10. Nazir, S. et al. Smoking Affects Bone Healing and Blood Perfusion. *Pakistan J. Med. Health Sci.* **16** (08), 580 (2022).
11. Ford, P. J. & Rich, A. M. Tobacco use and oral health. *Addiction* **116** (12), 3531–3540 (2021).

12. Organization, W. H. *WHO guideline for the clinical management of exposure to lead* (World Health Organization, 2021).
13. Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R. & Sadeghi, M. Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. *Front. Pharmacol.* **12**, 643972 (2021).
14. Alhumaimess, M. S. Sulfhydryl functionalized activated carbon for Pb (II) ions removal: Kinetics, isotherms, and mechanism. *Sep. Sci. Technol.* **55** (7), 1303–1316 (2020).
15. Bhattacharya, S. Essential trace metals as countermeasure for lead toxicity. *J. Environ. Pathol. Toxicol. Oncol.* ;**41**(2). (2022).
16. Ozoani, H. et al. Selenium and zinc alleviate hepatotoxicity induced by heavy metal mixture (cadmium, mercury, lead and arsenic) via attenuation of inflammo-oxidant pathways. *Environ. Toxicol.* **39** (1), 156–171 (2024).
17. Nagaraju, R., Kalahasthi, R., Balachandar, R. & Bagepally, B. S. Association between lead exposure and DNA damage (genotoxicity): systematic review and meta-analysis. *Arch. Toxicol.* **96** (11), 2899–2911 (2022).
18. Roy, Z., Bansal, R., Siddiqui, L. & Chaudhary, N. Understanding the role of free radicals and antioxidant enzymes in human diseases. *Curr. Pharm. Biotechnol.* **24** (10), 1265–1276 (2023).
19. Müller-Schüssele, S. J. et al. Chloroplasts require glutathione reductase to balance reactive oxygen species and maintain efficient photosynthesis. *Plant J.* **103** (3), 1140–1154 (2020).
20. Peterson, L. A. et al. Applying tobacco, environmental, and dietary-related biomarkers to understand cancer etiology and evaluate prevention strategies. *Cancer Epidemiol. Biomarkers Prev.* **29** (10), 1904–1919 (2020).
21. Addissouky, T. A. et al. Oxidative stress and inflammation: elucidating mechanisms of smoking-attributable pathology for therapeutic targeting. *Bull. Natl. Res. Centre* ;**48**(1). (2024).
22. Alkufi, A. A., Oleiwi, M. H. & Abojassim, A. A. Heavy metals in blood serum of smokers and non-smoking controls. *Curr. Anal. Chem.* **20** (3), 175–182 (2024).
23. Ho, K-J., Chen, T-H., Yang, C-C., Chuang, Y-C. & Chuang, H-Y. Interaction of smoking and lead exposure among carriers of genetic variants associated with a higher level of oxidative stress indicators. *Int. J. Environ. Res. Public Health*. **18** (16), 8325 (2021).
24. Dai, H. D., Hao, Y., Periera, M. & Michaud, T. Biomarkers of exposure to tobacco-related toxicants and oxidative stress among cancer survivors. *Nicotine Tob. Res.* **28** (3), 404–411 (2026).
25. Organization, W. H. *Exposure to lead: a major public health concern* (World Health Organization, 2023).
26. Gameli, P. S., Fleming, G., Walton, M. & Gluodenis, T. Human Health Effects from Exposure to Lead: A Review of the Current Literature. *Am. J. Biomedical Life Sci.* **10** (5), 135–145 (2022).
27. Diya, S. M. & Ibadi, E. A. Oxidative Stress, Inflammation and Diseases that Induced by Cigarette Smoke Toxicity. (2025).

28. Ullah, Z. et al. Impact of Cigarette Smoking on Blood Cadmium and Lead Levels and Its Association with Hematological Parameters in Adult Males of Samar Bagh, Pakistan. *J. Health Wellness Community Res.* :e39–e. (2025).
29. Collin, M. S. et al. Bioaccumulation of lead (Pb) and its effects on human: A review. *J. Hazard. Mater. Adv.* **7**, 100094 (2022).
30. JOSHI, B., SINGH, S., SHARMA, P. & MOHAPATRA, T. KUMAR P. Effect of Cigarette Smoking on Selected Antioxidant Enzymes and Oxidative Stress Biomarkers. *J. Clin. Diagn. Res.* ;**14**(10). (2020).
31. Chauhan, P., Reddy, S. S., Chokkanna, V. K., Singh, P. & Majumdar, K. Oxidant and antioxidant status among tobacco users: A cross-sectional study. *Natl. J. Maxillofacial Surg.* **14** (3), 444–449 (2023).
32. Pandi, A., Lal, V., Chakraborty, B., Kalappan, V. M. & LAL, P. A. N. D. I. A. V, et al. Evaluation of oxidative stress and antioxidant biomarkers in chronic cigarette smokers: a pilot study. *Cureus* ;**16**(5). (2024).
33. Verma, M. K., Paul, A. & Roy, M. Antioxidant defense: key mechanism of lead intolerance. Lead Toxicity Mitigation: Sustainable Nexus Approaches: Springer; pp. 263 – 75. (2024).
34. Lakka, N., Pai, B., Mani, M. S. & Dsouza, H. S. Potential diagnostic biomarkers for lead-induced hepatotoxicity and the role of synthetic chelators and bioactive compounds. *Toxicol. Res.* **12** (2), 178–188 (2023).
35. Parameswaran, S. et al. Comparative analysis of nicotine dynamics and antioxidant enzyme activity in high-tar and low-tar tobacco combustion users and healthy volunteers. *Biomed. Pharmacother.* **195**, 118987 (2026).
36. Nobari, H. et al. The effect of acute intense exercise on activity of antioxidant enzymes in smokers and non-smokers. *Biomolecules* **11** (2), 171 (2021).
37. Śniadach, J., Kicman, A., Michalska-Falkowska, A., Jończyk, K. & Waszkiewicz, N. Changes in concentration of selected biomarkers of exposure in users of classic cigarettes, e-cigarettes, and heated tobacco products—A narrative review. *Int. J. Mol. Sci.* **26** (5), 1796 (2025).
38. Darabseh, M. Z. et al. Fourteen days of smoking cessation improves muscle fatigue resistance and reverses markers of systemic inflammation. *Sci. Rep.* **11** (1), 12286 (2021).
39. Oladunjoye, Z., Adejumo, E., Ganiyu, A. & Quadri, J. Serum Glutathione Peroxidase and Superoxide Dismutase Levels in Young Adult Active Smokers and Non-Smokers in a Southwest Based Tertiary Institution. *SJMLS* ;**7**(4). (2022).
40. Bao, X. et al. Exposure to waterpipe smoke and blood heavy metal concentrations. *Environ. Res.* **200**, 111460 (2021).

Figures

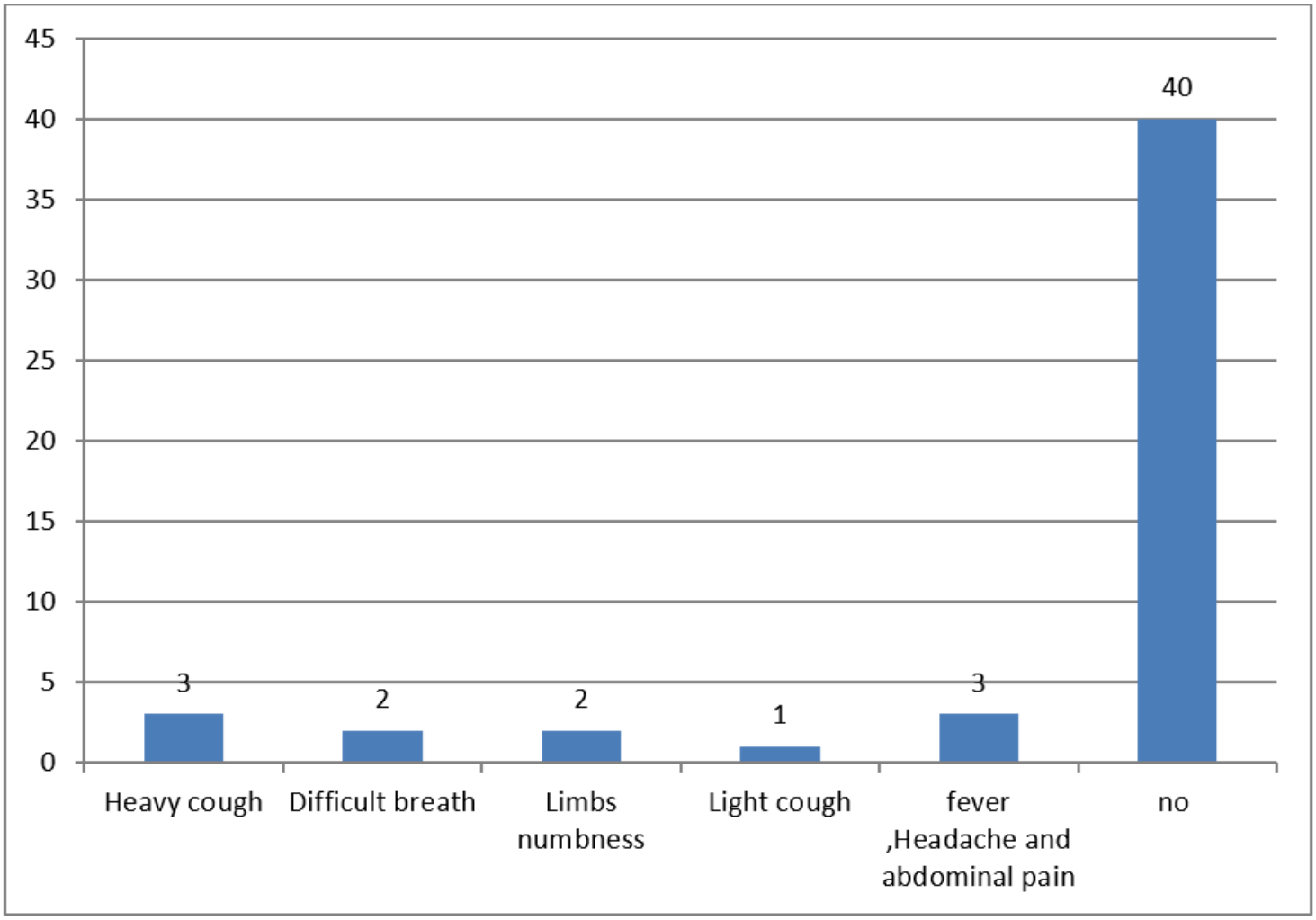


Figure 1

Distribution of symptoms among smokers