MJMR, Vol. 35, No. 3, 2024, pages (61-65).

Open Access
ISSN:2682-4558

Research Article

Evaluation of Nitric oxide level in serum of patients with lung diseases

Shereen Samy Gaber [1], Maggie M. Ramzy [1], Ahmed H Kasem[2], Hala Mohamed M.Marzouk[1], Bothina Ahmed Kamel[1]

[1] Biochemistry department, Faculty of Medicine, Minia University, Minia, Egypt
[2] Chest department, Faculty of Medicine, Minia University, Minia, Egypt

DOI: 10.21608/mjmr.2023.218850.1425

Abstract

Background: The respiratory diseases are major cause of disability and mortality, worldwide. Among the significant airborne pollutants affecting lung health, tobacco smoke stands out. Its detrimental effects include the promotion of inflammation, apoptosis, and oxidative stress in patients with chronic obstructive pulmonary disease (COPD) and lung cancer. Oxidative stress has widely contributed to diverse diseases, including COPD, and lung cancer. So there is an evidence suggested that excessive nitric oxide (NO) in lung tissue plays an important role in activation of platelet, stimulate chronic inflammation, and oxidative stress that leads to destruction of lung capillary endothelium and microangiopathy**.** The objective of this study was to examine the significant involvement of nitric oxide as a marker of oxidative stress related to inflammation and apoptosis in COPD and lung cancer patients. **Methods;** the levels of nitric oxide were measured using the ELISA method in serum samples from healthy controls (n=10), COPD patients (n=16), and lung cancer patients (n=16). **Results:** The results demonstrated a significant increase in nitric oxide levels in both the COPD and lung cancer groups compared to the healthy control group. **Conclusion:** In conclusion, these findings collectively suggest that elevated levels of nitric oxide are associated with oxidative stress in COPD and lung cancer patients and play a crucial role in the damage to lung epithelial cells and the development of both COPD and lung cancer.

.

Key words: Oxidative stress, Tobacco smoke, Nitric Oxide; COPD, lung cancer

Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the two types of free radicals that contribute to oxidative stress[1]. Smoking cigarettes is well-known to play a crucial role in developing lung cancer and COPD. It contains toxic chemical substances, such as reactive oxygen species (ROS) that induce airway inflammation and remolding which persists even after cigarette smoking cessation[2]. Reactive oxygen and nitrogen species (RNOS) and reactive oxygen species (ROS) are the driver of many pathways in both COPD and lung cancer. They may react with DNA, causing DNA damage that, if improperly repaired, can result in mutations. Moreover, RNOS and ROS activity can hinder mechanisms such as DNA repair and apoptosis that prevent mutation[3]. ROS including Superoxide and hydroxyl radicals as examples of radical species as well as single oxygen, hydrogen peroxide, hypochlorous acid, and ozone as examples of non-radical one. RNS includes nitric oxide and nitrogen dioxide[4]. Respiratory diseases continue to be a significant global health concern, contributing to high rates of mortality and disability worldwide. Millions of individuals worldwide suffer from Chronic Obstructive Pulmonary Disease (COPD), which is a preventable and treatable disease [4]. About

5% of all deaths (2.9 million) and 5% of disability-adjusted life years (DALYs) worldwide were caused by COPD [5]. It is a serious lung disease, characterized by persistent respiratory symptoms due to lung emphysema and small airway narrowing, and associated with chronic inflammatory response in the airways. Comorbidities and exacerbations determine the patient's overall severity[6]. Lung cancer is a respiratory tract cancer with high malignancy[7]. It is the main cause of cancer related death world-wide .The prognosis for the lung cancer cases remains poor, despite extraordinary advancements in clinical and experimental oncology in recent years. The 5-year overall survival rate is nearly 15%.which is unsatisfactory [8]. Primary lung cancers are usually classified into non-small cell lung cancer (NSCLC) with a percentage approximately 85% of all lung cancers and 15% of lung cancer cases are small cell lung cancer (SCLC), and the majority of NSCLC patients—nearly 80%—are diagnosed with advanced stages[9]. Oxidative stress markers, such as nitric oxide, and 8-isoprostane, and nitrogen oxides, are elevated in the respiratory tract, lungs, and blood of COPD patients[10]. The aim of this study was to investigate the significant involvement of nitric oxide as a potential marker of oxidative stress associated with inflammation and apoptosis in patients diagnosed with COPD and lung cancer. To accomplish this objective, the levels of nitric oxide were measured utilizing the enzyme-linked immunosorbent assay (ELISA) method. Understanding the intricate relationship between nitric oxide, oxidative stress, and respiratory diseases holds immense significance in uncovering the underlying mechanisms of disease progression and identifying potential therapeutic targets. The findings of this study have the potential to contribute to the growing body of knowledge in this field, offering valuable insights into the role of nitric oxide as a marker for oxidative stress in COPD and lung cancer.

Patients and methods

Group 1: Control Group

This group consisted of 10 healthy individuals who were carefully selected based on their confirmed good health status and the absence of any family history of lung disease. These individuals served as the control group for comparative analysis.

Group 2: COPD Patients

Sixteen patients diagnosed with chronic obstructive pulmonary disease (COPD) were included in this group. The diagnosis of COPD was made based on clinical evaluation at Minia Cardiothoracic University Hospital, and further confirmation was obtained through chest X-ray examinations.

Group 3: Lung Cancer Patients

Sixteen patients with lung cancer were included in this group. These individuals had not received any prior chemotherapy, radiation therapy, or other cancer treatments. The pathological features of the lung cancer samples were obtained from patients' medical records to ensure accurate classification.

Sample collection and storage:

Upon study registration, peripheral venous blood samples (5ml) from both patients and healthy individual were taken, and they were then kept at room temperature for 15–30 minutes to clot. Subsequently, the samples were subjected to centrifugation at a speed of 1000-2000 x g for 10 minutes, resulting in the separation of clear sera samples. To ensure their integrity for subsequent testing, all serum samples were appropriately kept in a freezer at a temperature of -80 °C.

Enzyme-linked Immunosorbent Assay

The concentration of human nitric oxide (NO) in serum was measured using the Human Nitric Oxide ELISA kit (SunLong Biotech Co., LTD) following the manufacturer's instructions. To prepare the standards, they were appropriately diluted according to the instructions provided with the ELISA kit. In each well of the ELISA plate, 10 µl of the serum sample and 40 µl of sample dilution buffer were added. One well was left empty to serve as a control. The plate was then incubated at 37°C for a period of 30 minutes. Following three rounds of washing, 50 µl of enzyme binding solution was added to each well, except for the blank well. The plate was again incubated at 37°C for 30 minutes. Subsequently, 50 µl of Chromogen Solution A and 50 µl of Chromogen Solution B were added to each well, mixed thoroughly, and incubated at 37°C for 15 minutes to induce color development. To stop the reaction, 50 µl of termination solution was added to each well. Finally, the absorbance of the samples was measured at a wavelength of 450 nm within 15

minutes after the addition of the stop solution. The results were calculated using the blank well as the baseline. A standard curve was generated using the Excel program to facilitate the quantification of the nitric oxide concentration in the serum samples.

Ethical consideration:
Ethical approval for the research project was obtained from the Research Ethics Committee of the Faculty of Medicine, Minia University, Egypt (Ethical permission No. 678:8/2020, Date: 25th August 2020). All participants in the study provided their informed consent prior to their inclusion in the research, ensuring compliance with ethical standards and safeguarding the rights and well-being of the participants.

Statistical analysis:
Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 20.0. Statistical comparisons of parametric quantitative data among the three groups were performed using the one-way analysis of variance (ANOVA) test. Subsequently, for further assessment of significance between individual pairs of groups, the Tukey's post hoc test was employed. The results were presented as the mean values accompanied by their corresponding standard error of mean for each group. A p-value less than 0.05 was considered statistically significant, indicating meaningful differences between the groups.

Results:
Measurement of serum Nitric oxide (NO) by ELISA:
The levels of nitric oxide were evaluated in all study groups utilizing the highly sensitive ELISA technique. The obtained results revealed a statistically significant variation in the concentration of serum nitric oxide among the different study groups. Specifically, the measurement of serum nitric oxide exhibited a noteworthy elevation in the COPD patient group in comparison to the control group, with a calculated p-value of 0.034. Furthermore, the lung cancer patient group demonstrated a significantly higher nitric oxide level in comparison to both the COPD patient group (p-value = 0.0001) and the control group (p-value = 0.0001), indicating a substantial increase in nitric oxide concentration (as shown in Table 1).

Table 1: The mean value ± SEM of nitric oxide serum of COPD and lung cancer patient

Group	Control group (mean±SEM) (N=10)	COPD patients (mean±SEM) (N=20)	Lung cancer patients (mean ± SEM) (N=20)	P value of COPD patients compared to control group	P value of lung cancer patients compared to control	P value of lung cancer patients compared to COPD patients
NO serum level	0.79±0.0 nmol /ml	1.25± 0.169	3.7 ± 0.192	0.034*	0.0001**	0.0001**

*; significance, **; highly significance

Discussion
The imbalance of the oxidation/antioxidant system brought on by the buildup of free radicals, especially reactive oxygen species (ROS) and reactive nitrogen species (RNS), is referred to as oxidative stress[11]. Oxidative stress contributed to different inflammatory related diseases, including pulmonary diseases, cardiovascular disease as well as many cancers including lung cancer[12]. Around 10% of adults have chronic obstructive pulmonary disease (COPD) which considered the most common respiratory non-communicable disease (NCD) [13]. The most common type of cancer in both men and women is lung cancer and it is still the leading cause of death from cancer globally[14]. Nitric oxide (NO) acts as a signaling molecule at low doses. Meanwhile at large amounts, NO induce cellular stress, DNA damage, mutagenesis, and apoptotic signaling pathways[15]. Additionally RNOS and ROS can increase the risk of infection and promote pulmonary inflammation[16]. In this report, we assessed Nitric oxide by ELISA technique to

detect its role in oxidative stress in serum of COPD and lung cancer patients.

The results in our study revealed that, serum NO level of COPD patients were increased significantly compared to the healthy group.

Similar to our result, another result has **revealed** that NO has been elevated in COPD lung tissue patients predominantly compare to healthy group[17].

Furthermore**, (Chen et al., 2014)**[18] found that NO level and NO synthase activity were increased in cultured Human bronchial epithelial cells (16HBE) treated with CSE. Moreover, total glutathione level was decreased**.**

In contrast to our results, another study found that NO level was dowenregulated in lung tissue samples and blood samples of patients with diabetic lung disease compared to normal healthy persons as well as iNOS mRNA expression level and iNOS protein expression were decreased in blood and lung tissue samples of diabetic lung disease patients[19].

Regarding evaluation of NO in lung cancer, our results showed that NO level was significantly increased compared to healthy control. In consistence with our results, another study found that exhaled NO and total nitrite have been increased significantly in lung cancer patients compared with healthy control people[20]**.** in the same line of our result , with employing of invivo measurements of NO and its reaction products, **(Nicola et al., 2021**)[21]showed that individuals with lung cancer have higher levels of NO and nitrite than healthy control participants.

In contrast to our result , **(Hamiza et al., 2012**) [22]has found that NO level were decreased during the transition of human colonic mucosa to polyps and then to carcinomas. Moreover, the amount of NOS protein and NOS enzyme activity which are responsible for endogenous production of NO were declined.

Conclusion

In conclusion, the results of this study support the notion that elevated levels of nitric oxide are closely linked to oxidative stress in patients with COPD and lung cancer. The findings indicate that nitric oxide plays a vital role in the damage inflicted upon lung epithelial cells, contributing to the development and progression of both COPD and lung cancer. These insights provide valuable information for understanding the underlying mechanisms involved in these respiratory diseases, and they may contribute to the development of novel therapeutic approaches targeting nitric oxide-mediated oxidative stress in the future.

Acknowledgements

I am greatly honored to express my gratitude to Dr. Salama Rabei Abdel Raheim Professor & Head of Biochemistry department, Faculty of Medicine, Minia University who was so cooperative, gave his continuous guidance.

Funding sources

This research did not receive any specific grant from funding agencies in the public commercial sectors.

References

1. Ďuračková, Z. Some current insights into oxidative stress. Physiological research. (2010); 59(4)
2. Hikichi, M., Mizumura, K., Maruoka, S., & Gon, Y. Pathogenesis of chronic obstructive pulmonary disease (COPD) induced by cigarette smoke. Journal of thoracic disease. (2019);11(Suppl 17), S2129
3. Wang, X., Shen, C., Zhu, J., Shen, G., Li, Z., & Dong, J. Long noncoding RNAs in the regulation of oxidative stress. Oxidative medicine and cellular longevity .Oct;2019.; 2019.
4. Thimmulappa, R. K., Chattopadhyay, I., & Rajasekaran, S. Oxidative stress mechanisms in the pathogenesis of environmental lung diseases. Oxidative Stress in Lung Diseases.(2020); Volume 2, 103-137.
5. Phillips, D. B., Elbehairy, A. F., James, M. D., Vincent, S. G., Milne, K. M., De-Torres, J. P., ... & O'Donnell, D. E. Impaired ventilatory efficiency, dyspnea, and exercise intolerance in chronic obstructive pulmonary disease: results from the CanCOLD study. American Journal of Respiratory and Critical Care Medicine .(2022);205(12), 1391-1402.
6. Gayle, A. Understanding the burden of COPD mortality: An investigation usingUK electronic healthcare record data. (2022).

7. Miller, K. D., Nogueira, L., Mariotto, A. B., Rowland, J. H., Yabroff, K. R., Alfano, C. M., . . . Siegel, R. L. Cancer treatment and survivorship statistics, 2019. CA: a cancer journal for clinicians. (2019); 69(5), 363-385.
8. Arenberg, D. Update on screening for lung cancer. Translational lung cancer research. (2019); 8(Suppl 1), S77.
9. Hiddinga, B. I., Raskin, J., Janssens, A., Pauwels, P., & Van Meerbeeck, J. P. Recent developments in the treatment of small cell lung cancer. European Respiratory Review. (2021); 30(161).
10. McGuinness, A. J. A., & Sapey, E. Oxidative stress in COPD: sources, markers, and potential mechanisms. Journal of clinical medicine. (2017); 6(2), 21.
11. Miller, K. D., Nogueira, L., Devasia, T., Mariotto, A. B., Yabroff, K. R., Jemal, A., ... & Siegel, R. L. Cancer treatment and survivorship statistics, 2022. CA: a cancer journal for clinicians. (2022);72(5), 409-436.
12. Patergnani, S., bouhamida, E., leo, S., pinton, P. & rimessi, A. Mitochondrial oxidative stress and "mito-inflammation": actors in the diseases. Biomedicines. 2021; 9, 216.
13. Halpin, D., celli, B., criner, G., frith, P., varela, L., salvi, S., vogelmeier, C., chen, R., mortimer, K. & montes de oca, M. The gold Summit on chronic obstructive pulmonary disease in low-and middle-income countries. The International Journal of Tuberculosis and Lung Disease. 2019;23, 1131-1141.
14. Planchard, D., Popat, S. T., Kerr, K., Novello, S., Smit, E. F., Faivre-Finn, C., ... & Peters, S. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. (2018);29, iv192-iv237.
15. Li, C. Q., Kim, M. Y., Godoy, L. C., Thiantanawat, A., Trudel, L. J., & Wogan, G. N. Nitric oxide activation of Keap1/Nrf2 signaling in human colon carcinoma cells. Proceedings of the National Academy of Sciences. (2009); 106(34), 14547-14551.
16. Durham, A., & Adcock, I. The relationship between COPD and lung cancer. Lung Cancer. (2015); 90(2), 121-127.
17. Paraskakis, E., Vergadi, E., Chatzimichael, A., & Bush, A. The role of flow-independent exhaled nitric oxide parameters in the assessment of airway diseases. Current topics in medicinal chemistry. (2016); 16(14), 1631-1642.
18. Chen, L., Wang, T., Guo, L., Shen, Y., Yang, T., Wan, C., ... & Wen, F. Overexpression of RAGE contributes to cigarette smoke-induced nitric oxide generation in COPD. Lung. (2014); 192, 267-275.
19. Li, P., Zhang, N., Ping, F., Gao, Y., & Cao, L. lncRNA SCAL1 inhibits inducible nitric oxide synthase in lung cells under high‐glucose conditions. Experimental and Therapeutic Medicine. (2019); 18(3), 1831-1836.
20. Masri, F. A., Comhair, S. A., Koeck, T., Xu, W., Janocha, A., Ghosh, S., . . . Stuehr, D. J. Abnormalities in nitric oxide and its derivatives in lung cancer. American journal of respiratory and critical care medicine. (2005); 172(5), 597-605.
21. Nicola, S., Ridolfi, I., Rolla, G., Filosso, P., Giobbe, R., Boita, M., . . . Brussino, L. IL-17 promotes nitric oxide production in non-small-cell lung cancer. Journal of Clinical Medicine. (2021); 10(19), 4572.
22. Hamiza, O. O., Rehman, M. U., Tahir, M., Khan, R., Khan, A. Q., Lateef, A., . . . Sultana, S. Amelioration of 1, 2 Dimethylhydrazine (DMH) induced colon oxidative stress, inflammation and tumor promotion response by tannic acid in Wistar rats. Asian Pacific Journal of Cancer Prevention. (2012); 13(9), 4393-4402