Heliyon 10 (2024) e31188

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

DO disease stages affect oxidative stress in stable COPD?

Murat Yalcinsoy [1], Aysegul Beykumul [2,*], Gazi Gulbas [2], Ahmet Kadir Arslan [3], Salim Neselioglu [4]

[1] Department of Pulmonary Medicine, Inonu University Medical Faculty, Turgut Ozal Medical Center, Malatya, Turkey
[2] Department of Pulmonary Rehabilitation, Inonu University Medical Faculty, Turgut Ozal Medical Center, Malatya, Turkey
[3] Department of Biostatistics, Inonu University, Malatya, Turkey
[4] Department of Biochemistry, Yıldırım Beyazıt University Medical Faculty, Ankara, Turkey

ARTICLE INFO

Keywords:
COPD
Oxidative stress
COPD stages
Thiol
Disulfide

ABSTRACT

Background: Detection of oxidative stress level may lead us to understand the pathogenesis of COPD better and to search for new treatments. Oxidative stress levels have also been shown to be elevated in stable COPD patients. We aimed to investigate whether the stage of COPD affects the severity of inflammation-induced oxidative stress in patients with stable COPD.
Methods: Between June 2019 and March 2020, all consecutive patients admitted to COPD-specific outpatient clinics were included. Patients were classified A, B, and E according to the GOLD guideline.
Results: The median age of 98 patients (Male: 92 (93.9 %)) was 65 (min-max: 49–86). A statistically significant difference was found between the groups in FEV1, FVC, and FEV1/FVC ($p < 0.001$). age, and thiols ($r = -0.168$, $p = 0.049$; $r = -0.184$, $p = 0.035$) and DS ($r = -0.209$, $p = 0.019$) were found to be negatively correlated at a low level. When adjusted for age, oxidative stress parameters were similar between stages.
Conclusion: No difference between stages and oxidative stress parameters according to GOLD classification in stable COPD patients. Our results may be a guide for not using anti-inflammatory therapy except for attacks.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is defined by Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease (GOLD) as" a common, preventable, and treatable disease characterized by persistent airflow limitation and respiratory symptoms due to airway and/or alveolar disruption, caused by severe exposure to harmful particles or gases and host factors, including abnormal lung development" [1]. The pathogenesis of COPD occurs due to the body's exposure to inhaled harmful particles and gases. Although exposure to tobacco smoke is the leading risk factor in genetically susceptible individuals, exposures due to environmental, occupational, and indoor air pollution are important risk factors contributing to the development of COPD. The abnormal inflammatory response that develops due to exposure to cigarette smoke and harmful particles, which are the leading causes of COPD pathogenesis, disrupts all mechanisms. Therefore, the developing inflammation causes damage to the lung tissue and airways [2].

* Corresponding author.
E-mail addresses: mrtyalcinsoy@yahoo.com (M. Yalcinsoy), abeykumul@yahoo.com (A. Beykumul), gazigulbas@yahoo.com (G. Gulbas), ahmetkadirarslan@gmail.com (A.K. Arslan), salim_neselioglu@hotmail.com (S. Neselioglu).

https://doi.org/10.1016/j.heliyon.2024.e31188
Received 23 January 2024; Received in revised form 9 May 2024; Accepted 12 May 2024
Available online 18 May 2024

Reactive oxygen species (ROS) is a collective term that includes a wide variety of free oxygen radicals such as superoxide anion (O2-) and hydroxyl radical (OH-), as well as unpaired electron-free oxygen derivatives such as hydrogen peroxide (H2O2). During normal metabolic processes, ROS formation takes place continuously in every cell. Increased ROS levels are associated with inflammatory reactions such as transcriptional alteration, signal transduction, or gene expression of pro-inflammatory mediators [3]. In the respiratory system, ROS may be exogenous, originating from inhaled gaseous or particulate agents such as air pollutants, cigarette smoke, high altitude hypoxia, and certain occupational dusts in the environment. Alternatively, ROS may be endogenously generated in the context of defence mechanisms against infectious agents such as bacteria, viruses, or pathogens. Thiols are organic compounds that plays a pivotal role in preventing the formation of any oxidative stress state in cells. Thiols and disulfides have roles in stabilizing the structures of proteins, regulating their functions, enzyme functions, receptors, transporters, Na–K channels, and transcription. It is involved in dynamic thiol/disulfide equilibrium state, antioxidant defense, detoxification, apoptosis, regulation of enzyme activities, transcription, and cellular signal transduction mechanisms [4].

Detection of oxidative stress level in patients with COPD may guide us to understand COPD's pathogenesis better and seek new treatments oxidative stress parameters are known to increase in COPD exacerbation compared to the stable period. Since oxidative stress is an ongoing systemic response with autoimmunity, it has been shown to be higher in stable COPD patients than in the control group. However, oxidative stress parameters between stages have not been studied in stable COPD patients [5]. We aimed to investigate whether the stage of COPD affects the severity of inflammation-induced oxidative stress in patients with stable COPD.

2. Material and method

The study was conducted between June 2019 and March 2020 at the Department of Chest Diseases of University hospital. Approval was obtained from the university's interventional ethics committee for this randomized, prospective, interventional study (~~approval~~ local ethical number: 2019/106). Prior to the procedure, patients were provided with comprehensive information and were required to sign a consent form.

3. Patient population

Consecutive patients who applied to COPD specific outpatient clinic with COPD and agreed to participate in the study were included. Exclusion criteria; COPD exacerbation, non-COPD respiratory disease, asthma-COPD overlap syndrome (ACOS), malignancy, kidney disease, patients who received steroid treatment, previous surgery in the last month.

The diagnosis and classification of COPD were made according to the GOLD criteria. Patients were classified according to GOLD combined assessment as A, B, and E [1]. The definition of stable COPD is that the patient's cough, expectoration, and shortness of breath are in stable condition or just show mild symptoms, or the condition is basically restored to the state before acute exacerbation [6].

4. Study design

Whole blood, biochemistry, C-reactive protein, and chest X-rays were examined in routine outpatient evaluations. Medication arrangements were made for the examined patients by taking their anamnesis. A pulmonary function test (PFT) was performed with a

Table 1
Demographic features of the participants.

Age (median)	65 (min-max: 49–86)
BMI (kg/m2) mean ± SD	25.36 ± 4.13
PFT	
FEV1 (ml)	1376.33 ± 676.17
FVC (ml)	2423.47 ± 845.30
FEV1/FVC	54.73 ± 12.83
GOLD Stages	n (%)
A	14 (14.3)
B	31 (31.6)
E	53 (54.1)
GENDER	
Female	6 (6.1)
Male	92 (93.9)
MMRC	
1	19 (19.4)
2	48 (49.0)
3	16 (16.3)
4	15 (15.3)
Total	**98**

BMI: Body Mass Index; PFT: Pulmonary Function Test; GOLD: Global Initiative for Chronic Obstructive Lung Disease; mMRC: modified Medical Research Dyspnea Questionnaire.

Vmax (Viasys-Healthcare-2007 GmbH, Höchberg, Germany) brand device. In addition to these evaluations, four ccs of blood were taken into an EDTA tube to determine oxidative stress levels. These samples were centrifuged at 4000 rpm for 10 min (Hettichzentrifugen, Rotina 380, Germany). plasma was stored at −80 °C and they were sent to Biochemistry laboratory (Yıldırım Beyazıt University) with dry ice. Dynamic thiol/Disulfide balance was evaluated (an automated clinical chemistry analyzer – Roche-Cobas 501, Mannheim, Germany). An increase in Native thiol (NT), Total thiol (TT) values, and a decrease in Disulfide (DS)value were assessed as a decrease for oxidative stress level [7].

5. Statistical analysis

The variables employed in the study were tabulated according to the following parameters: median (interquartile range), frequency, and percentage, according to measurement levels. The assumption of normal distribution for numerical variables was evaluated using the Shapiro-Wilk test. Kruskal-Wallis H test was used for comparisons between groups in terms of quantitative variables and Pearson chi-square tests were used for qualitative variables. Conover test was used for multiple comparisons after Kruskal-Wallis H test. The correlation between quantitative variables was analyzed with Spearman's rho correlation coefficient. $p \leq 0.05$ was accepted as statistical significance level. IBM SPSS Statistics 27 program was used in the analysis.

6. Results

The median age of 98 patients (F/M: 6 (6.1 %)/92 (93.9 %)) included in the study was 65 (min-max: 49–86). Demographic values are presented in Table 1.

Oxidative stress parameters and physiological parameters were compared according to GOLD stages. Among the oxidative stress parameters, only the disulfide level was significantly lower in group E compared to the other groups (p = 0.03811). Considering the physiological parameters, a statistically significant difference was found between the groups in forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), and FEV1/FVC ratio ($p < 0.001$), and respiratory capacity decreased as the GOLD stage increased (Table 2). Smoking was similar across GOLD stages (p = 0.304) (Table 3).

The correlation of oxidative stress parameters with body mass index (BMI), age, GOLD stages, and PFT values was analyzed (Table 4). There was a moderate negative correlation between the GOLD stage and TT (r = −0.169, p = 0.048) and a low negative correlation between DS (r = −0.234, p = 0.010). The correlation between PFT values and indices, FEV1 and FVC were low (FEV1: r = 0.293, p = 0.002, FVC: r = 0.193, p = 0.028), FEV1/FVC was moderate (r = 0.333, p < 0.001), FVC was found to be associated with DS at a low level (r = 0.174, p = 0.043), age, thiols (r = −0.168, p = 0.049; r = −0.184, p = 0.035) and DS (r = −0.209, p = 0.019) were found to be negatively correlated at a low level. The correlations between BMI, GOLD stages, pulmonary function tests and oxidative stress parameters after excluding the effect of age are shown in Table 5.

7. Discussion

It is known that oxidative stress in the pathogenesis of COPD starts with endogenous or exogenous stimuli and continues with autoimmunity even if the stimuli are discontinued. However, the level of oxidative stress between the stages is unknown. To our knowledge, this study is the first study to investigate oxidative stress levels with thiols according to the stage in stable COPD patients. According to GOLD classification in stable COPD patients, our study showed no difference between stages and oxidative stress parameters. In addition, clinical studies have shown an age-related shift in plasma thiol/disulfide redox couples in some age-related diseases such as cardiovascular diseases, diabetes, and rheumatoid arthritis [8,9]. The reason for the age-related change in thiol/disulfide balance is not yet fully known. However, in our study, similar to the literature, an age-related change in oxidative stress was detected [10].

Oxidative stress studies have shown that free oxygen radicals, which arise as a result of defense mechanisms against inhaled toxic

Table 2
Comparison of the difference between groups in parameters according to COPD stages.

Variable*	GOLD**			*p*
	A	B	E	
Age	64.5 ab (8.75)	61a (9.5)	68b (12)	**0,00297**
FVC (lt)	3065a (725)	2320a (1020)	2000b (1000)	**0,00102**
FEV1 (lt)	2065a (957.5)	1450b (885)	970c(780)	**<0,001**
FEV1/FVC	63a (11.21)	56 ab (18.76)	49.13b (20.73)	**0,01164**
nativethiol	379 (84.75)	354 (120.5)	336 (115)	0,29266
totalthiol	419 (93.5)	401 (120)	370 (124)	0,2109
disulfide	20.5a (5)	20.5a (6.75)	17.5b (5.5)	**0,03811**
disulfide_nt	5.667 (1.711)	5.159 (1.822)	5.276 (1.701)	0,50057
disulfide_tt	5.09 (1.366)	4.677 (1.481)	4.773 (1.388)	0,50057
nt_tt	89.821 (2.732)	90.646 (2.963)	90.455 (2.776)	0,50057

*: Variables are summarized as 'median (interquartile ranges)'.
**: There is a statistically significant difference in group categories that do not contain the same letter (APA).

Table 3
Smoking levels according to COPD stages.

			GOLD			Total	*p*
			A	B	E		
Quit_smoking							
	never-smoking	n	1	1	1	3	0,304
		%	7,10 %	3,20 %	1,90 %	3,10 %	
	smoking	n	6	7	6	19	
		%	42,90 %	22,60 %	11,30 %	19,40 %	
	less than 1 year	n	1	3	5	9	
		%	7,10 %	9,70 %	9,40 %	9,20 %	
	1–3 years	n	2	7	8	17	
		%	14,30 %	22,60 %	15,10 %	17,30 %	
	4–6 years	n	0	4	7	11	
		%	0,00 %	12,90 %	13,20 %	11,20 %	
	7–9 years	n	0	1	7	8	
		%	0,00 %	3,20 %	13,20 %	8,20 %	
	10 years and above	n	4	8	19	31	
		%	28,60 %	25,80 %	35,80 %	31,60 %	
	Total	n	14	31	53	98	
		%	100,00 %	100,00 %	100,00 %	100,00 %	

GOLD: Global Initiative for Chronic Obstructive Lung Disease.

Table 4
Correlations of parameters with thiols, disulfide, and indices.

	NT		TT		DS		DS/NT		DS/TT		NT/TT	
	rho	*p*	rho	*p*	rho	*p*	rho	*p*	rho	*p*	rho	*p*
GOLD	−0.152	0.068	−0.169	**0.048**	−0.234	**0.010**	−0.080	0.218	−0.080	0.218	0.080	0.218
FEV1	−0.126	0.109	−0.106	0.150	0.164	0.053	0.293	**0.002**	0.293	**0.002**	−0.293	**0.002**
FVC	−0.043	0.336	−0.026	0.399	0.174	**0.043**	0.193	**0.028**	0.193	**0.028**	−0.193	**0.028**
FEV1/FVC	−0.210	**0.019**	−0.190	**0.030**	0.090	0.190	0.333	**<0.001**	0.333	**<0.001**	−0.333	**<0.001**
BMI	−0.136	0.091	−0.135	0.093	−0.052	0.305	0.061	0.275	0.061	0.275	−0.061	0.275
Age	−0.168	**0.049**	−0.184	**0.035**	−0.209	**0.019**	−0.020	0.421	−0.020	0.421	0.020	0.421

GOLD: Abbreviations: GOLD: Global strategy for the diagnosis, management, and prevention of Chronic Obstructive Lung Disease, BMI: Body mass index, NT: Native thiol, TT: Total thiol, DS: Disulfide, DS/NT: Index I, DS/TT: Index II, NT/TT: Index III.

particles, cause oxidative stress in smokers [11,12]. However, it is known that smoking cessation slows down the decline in lung function and decreases the endogenous mechanisms that cause oxidative stress six months after smoking cessation [13]. Obesity also triggers chronic inflammation and increased oxidative stress as a result of excess fat accumulation in individuals [14]. Since smoking and BMI were similar between groups in our study, COPD-specific oxidative stress could be evaluated. Gender distribution in COPD patients is usually 1.5–2 times in favor of male gender [15]. In our study, the majority of our patients were male. Since consecutive patients were recruited in the outpatient clinic, gender distribution was not interfered with. Although gender-related differences in sensitivity in disease occurrence have been reported, we do not think that they affect the results of our study since there is no clear information on oxidative stress responses.

A study performed with COPD and healthy controls staged as II, III, and IV according to the GOLD spirometric stage showed that the oxidative stress level increased with the progression of COPD [16]. In contrast, a separate study involving 50 patients with COPD and 33 healthy controls demonstrated that individuals with thiols exhibited lower thiol levels than control group [17]. In another study, no significant differences were observed in thiol levels between the groups of patients with asthma, COPD, and ACOS (Asthma-COPD overlap syndrome) [18]. In a study conducted with cement workers, it was found that pulmonary function test (PFT) values were negatively associated with oxidative stress [19].

In our study, pulmonary function values showed a negative correlation with stage in accordance with the literature. Oxidative stress parameters showed a negative correlation in the first correlation assessment. When the age factor was excluded, there was no difference between the stages. According to these results, we suggest that COPD composite staging may be more valuable than pulmonary function tests, especially in anti-inflammatory therapy.

The limitations of our study are that it is single-centered, the stages of COPD are not evenly distributed when divided into subgroups, and diets that may affect this are not considered when evaluating oxidative balance. The strength of our study is that it was performed in a COPD-specific outpatient clinic, and it was the first study on this subject.

In conclusion, we found that oxidative stress parameters were not different in stable COPD patients according to gold stages. We think that combined staging instead of functional staging may be important in the treatment management of stable patients. In addition, increasing antioxidant capacity with external antioxidants (such as vitamins A, C, E) and pulmonary rehabilitation may prevent attacks in all stable patients regardless of stage. It also protects from the use of unnecessary treatments and possible side

Table 5
Correlations of parameters with thiols, disulfide, and indices after excluding the age effect.

Control variable	Variables	Variables											
		NT		TT		DS		DS/NT		DS/TT		NT/TT	
		PCC	*p*	PCC	*p*	PCC	*p*	PCC	*p*	PCC	*p*	PCC	*p*
Age	**BMI**	0,043	0,674	0,035	0,734	−0,063	0,541	−0,11	0,285	−0,109	0,29	0,109	0,29
	GOLD	−0,098	0,341	−0,113	0,271	−0,214	0,035	−0,103	0,318	−0,102	0,322	0,102	0,322
	FVC	−0,053	0,607	−0,038	0,709	0,123	0,23	0,172	0,093	0,172	0,093	−0,172	0,093
	FEV1	−0,145	0,156	−0,126	0,217	0,114	0,265	0,255	**0,012**	0,255	**0,012**	−0,255	**0,012**
	FEV1/FVC	−0,29	**0,004**	−0,274	**0,007**	0,003	0,978	0,301	**0,003**	0,3	**0,003**	−0,3	**0,003**

Abbreviations: GOLD: Global strategy for the diagnosis, management, and prevention of Chronic Obstructive Lung Disease, BMI: Body mass index, NT: Native thiol, TT: Total thiol, DS: Disulfide, DS/NT: Index I, DS/TT: Index II, NT/TT: Index III, PCC: Partial Correlation Coefficient.

effects.

Funding

None.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Murat Yalcinsoy: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Aysegul Beykumul:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Gazi Gulbas:** Writing – review & editing, Conceptualization. **Ahmet Kadir Arslan:** Formal analysis, Data curation. **Salim Neselioglu:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis.

Declaration of competing interest

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

Acknowledgment

We would like to express our gratitude to Prof. Dr. Ozcan Erel for providing us with access to the laboratory facilities.

References

[1] Global strategy for diagnosis, management, and prevention of COPD. The global initiative for chronic obstructive lung diseases (GOLD) 2023 report. https://goldcopd.org/2023-gold-reports.
[2] P.K. Jeffrey, Remodelling in asthma and chronic obstructive lung disease, Am. J. Respir. Crit. Care Med. 164 (2001) S28–S38, https://doi.org/10.1164/ajrccm.164.supplement_2.2106061.
[3] I. Rahman, Oxidative stress in pathogenesis of chronic obstructive pulmonary disease: cellular and molecular mechanisms, Cell Biochem. Biophys. 43 (2005) 167–188, https://doi.org/10.1385/CBB:43:1:167.
[4] P.J. Barnes, Cellular and molecular mechanisms of chronic obstructive pulmonary disease, Clin. Chest Med. 35 (1) (2014) 71–86, https://doi.org/10.1016/j.ccm.2013.10.004.
[5] L.M. Kopcnovic, A.M. Domijan, K. Posavac, I. Cepelak, T.Z. Grubisic, L. Rumora, Systemic redox imbalance in stable chronic obstructive pulmonary disease, Biomarkers 21 (8) (2016) 692–698, https://doi.org/10.3109/1354750X.2016.1172110.
[6] Global strategy for diagnosis, management, and prevention of COPD. The global initiative for chronic obstructive lung diseases (GOLD) 2020 report. https://goldcopd.org/2020-gold-reports.
[7] O. Erel, S. Neselioglu, A novel and automated assay for thiol/disulphide homeostasis, Clin. Biochem. 47 (18) (2014 Dec) 326–332, https://doi.org/10.1016/j.clinbiochem.2014.09.026.
[8] E. Gamble, D.C. Grootendorst, K. Hattotuwa, T. O'Shaughnessy, F.S.F. Ram, Y. Qiu, J. Zhu, A.M. Vignola, C. Kroegel, F. Morell, I.D. Pavord, K.F. Rabe, P. K. Jeffery, N.C. Barnes, Airway mucosal inflammation in COPD is similar in smokers and ex-smokers: a pooled analysis, ERJ 30 (2007) 467–471, https://doi.org/10.1183/09031936.00013006.
[9] W.G. Kirlin, J. Cai, S.A. Thompson, D. Diaz, T.J. Kavanagh, D.P. Jones, Glutathione redox potential in response to differentiation and enzyme inducers, Free Radic. Biol. Med. 27 (11–12) (1999) 1208–1218, https://doi.org/10.1016/s0891-5849(99)00145-8.
[10] P. Maher, The effects of stress and aging on glutathione metabolism, Ageing Res. Rev. 4 (2005) 288–314, https://doi.org/10.1016/j.arr.2005.02.005.
[11] W. Domej, K. Oetti, W. Renner, Oxidative stress and free radicals in COPD implications and relevance for treatment, Int. J. Chronic Obstr. Pulm. Dis. 17 (9) (2014 Oct) 1207–1224, https://doi.org/10.2147/COPD.S51226.
[12] P.J. Barnes, Reactive oxygen species, in: P.J. Barnes, J.M. Drazen, S.I. Rennard, N.C. Thomson (Eds.), Asthma and COPD: Basic Mechanisms and Clinical Management, second ed., 9780123740014 Elsevier, 2008.
[13] J.E. Repine, A. Bast, I. Lankhorst, Oxidative stress in chronic obstructive pulmonary disease. Oxidative Stress Study Group, Am. J. Respir. Crit. Care Med. 156 (1997) 341–357, https://doi.org/10.1164/ajrccm.156.2.9611013.
[14] S. Musaad, E.N. Haynes, Biomarkers of obesity and subsequent cardiovascular events, Epidemiol. Rev. 29 (2007) 98–114, https://doi.org/10.1093/epirev/mxm005.
[15] T.A. Perez, E.G. Castillo, J. Ancochea, M.T. Pastor Sanz, P. Almagro, P. Martínez-Camblor, M. Miravitlles, et al., Sex differences between women and men with COPD: a new analysis of the 3CIA study, Respir. Med. 171 (2020) 106105, https://doi.org/10.1016/j.rmed.2020.106105.
[16] Y.D. Torres-Ramos, M.L. Garcia-Guillen, I.M. Olivares-Corichi, J.J. Hicks, Correlation of plasma protein carbonyls and C-reactive protein with GOLD stage progression in COPD patients, Open Respir. Med. J. 14 (3) (2009) 61–66, https://doi.org/10.2174/1874306400903010061.
[17] E. Erden, S. Motor, I. Ustun, M. Demırkose, R. Yuksel, R. Okur, S. Oktar, Y. Yakar, S. Sungur, C. Gokce, Investigation of Bisphenol A as an endocrine disruptor total thiol, malondialdehyde and C-reactive protein levels in chronic obstructive pulmonary disease, Eur. Rev. Med. Pharmacol. Sci. 18 (2014) 3477–3483. PMID: 25491624.
[18] E. Babaoglu, H. Kilic, H. Hezer, O. Dag, E. Parlak, A. Senturk, A. Karalezli, M. Alisik, O. Erel, H.C. Hasanoglu, Comparison of thiol/disulphide homeostasis parameters in patients with COPD, asthma and ACOS, Eur. Rev. Med. Pharmacol. Sci. 20 (8) (2016) 1537–1543. PMID: 27160126.
[19] S.A. Shanshal, H.K. Al-Qazaz, Spirometric outcomes and oxidative stress among cement factory workers: results from a cross-sectional study, J. Occup. Environ. Med. 62 (10) (2020) e581–e585, https://doi.org/10.1097/JOM.0000000000001991.