Conference Paper

The Effects of Allopurinol on Glutathione Sulfhydryl (GSH) Serum Level, %FEV$_1$, Six Minute Walking Test, and CAT Score of Chronis Obstructive Pulmonary Disease Patients

YS Sutanto, Suradi, and Samuel

Department of Pulmonology and Respiratory Medicine, Medical Faculty of Universitas Sebelas Maret / Dr. Moewardi General Hospital Surakarta

Corresponding Author:
YS Sutanto
research.ustpostgrad@gmail.com

Received: 23 February 2019
Accepted: 6 March 2019
Published: 25 March 2019

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the ICO-HELICS Conference Committee.

Abstract

Introduction: Stress oxidative in chronic obstructive pulmonary disease (COPD) impaired striated muscle thus worsening COPD clinical symptoms. Allopurinol as antioxidant reduces stress oxidative in COPD so it can improve exercise capacity and clinical symptoms of COPD patients. The aims of this study were to analyze the effect of allopurinol on glutathione sulfhydryl (GSH) serum level, %FEV$_1$, six-minute walking test (6MWT), and COPD assessment test (CAT) score of COPD group C and D patients.

Methods: This was a pre and post test group clinical trials held in pulmonology outpatient clinic in Dr. Moewardi general hospital, Surakarta from January to February 2018 using purposive sampling. The COPD group C and D patients were categorized as intervention group which received allopurinol 300 mg/day for four weeks and a control group which did not receive allopurinol. Glutathione sulfhydryl serum level, %FEV$_1$, 6MWT, and CAT score were measured at baseline and after four weeks in both groups.

Results: A total of 37 stable COPD group C and D patients were included in this study. The intervention group showed decreased of GSH level (52.58 ± 38.39) µg/ml and CAT score (10.37 ± 4.46) which were statistically significant compared to control group ($p<0,05$) while decreased of %FEV$_1$ in intervention group (6.91 ± 10.60) and control group (8.43 ± 11.26) were not significant statistically ($p=0.94$). The intervention group also showed a significant increase of 6MWT ($p=0.005$) while the control group showed no significant increase ($p=0.109$); however, the 6MWT differences in both groups were not significant with $p=0.057$.

Conclusion: Allopurinol decreased GSH serum level, did not influence %FEV$_1$, increased 6MWT, and decreased CAT score of stable COPD group C and D patients.

Keywords: COPD, allopurinol, GSH, %FEV$_1$, 6MWT, CAT

1. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a pulmonary disease with increasing prevalence due to increased harmful gas exposure and longer life expectancy. Male

gender, smoker, and age over forty are more frequent to be affected by COPD. Cigarette smoke or noxious agents is the primary cause of COPD [1-3].

Standard medical therapy for COPD which has been available so far is bronchodilators as well as anti-inflammation drugs, and these have not covered the whole COPD pathogenesis process so that it was not able to improve respiratory and skeletal muscle functions as well as exercise capacity of COPD patients. Giving antioxidant as adjuvant therapy is expected to be able to improve the effectiveness of stable COPD management. The origin of cellular oxidant sources are from mitochondrial respiration, nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase, dan xanthine oxidase (XO), while the sources come from the environment are cigarette smoke, noxious agents, and air pollution. An oxidant such as reactive oxygen species (ROS) mediates inflammation process resulting in both damage and change of respiratory tract structure so that respiratory tract obstruction and respiratory symptoms occur [2,4-7]. Xanthine oxidase functions to change hypoxanthine into xanthine and later changes it to uric acid. Xanthine oxidase enzyme oxidizes hypoxanthine with the support of oxygen and releases ROS specifically superoxidant which then changed into hydrogen peroxide (H_2O_2) causing endothelium dysfunction and muscle tissue injury. Glutathione sulfhydryl (GSH) is an endogenous antioxidant which originates from liver and it is subsequently distributed through blood then binds to ROS to form glutathione disulfide (GSSG). Muscle injury due to ROS and hypoxemia secondary to chronic respiratory tract obstruction promote skeletally and diaphragm muscle dysfunction as well as shortness of breath [5, 8, 10].

Stable COPD patients classified into 4 groups which are A, B, C, and D. The quality of life (QoL) of COPD patients primarily group C and D are mostly low or decreased due to shortness of breath and daily activity dysfunction result from decline in breathing and skeletal muscle's capability while the QoL of group A and B are a little bit better. Chronic obstructive pulmonary disease assessment test (CAT) is used to assess clinical symptoms while six minutes walking test (6MWT) is used to evaluate exercise capability of stable COPD patients. Currently, the existing standard medical therapy for COPD has not fully achieved the aim of COPD management especially in reducing shortness of breath and improving exercise capacity as well as ameliorating QoL of COPD patients [1, 2, 11]. Allopurinol, which until now has been known as anti hyperuricemia drug, can be used as an additional medicine apart from standard therapy for stable COPD. Allopurinol which is XO inhibitor is an antioxidant to prevent the formation of ROS which can be detected via GSH serum level as well as to reduce muscle morphology damage specifically edema in muscle fibers and mitochondria. Therefore giving this additional drug is expected to

reduce skeletal muscle tissue damage and to boost exercise capacity of stable COPD patients. The dose of allopurinol as an antioxidant is 300 mg per day as a single or split dose [9, 12].

This study aimed to investigate the effects of allopurinol as oxidative stress regulator on GSH serum level, percentage of forced expiratory volume in one second (%FEV_1), 6MWT, and CAT score of stable COPD patients group C and D. All patients were still given standard therapy for COPD according to guideline Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2017.

2. Methods

This was a pre and post test quasi-experimental study which was conducted in pulmonology outpatient clinic of Dr. Moewardi hospital, Surakarta from January to February 2018 by using purposive sampling. This study involved 38 stable COPD patients group C and D who were willing to join this study by signing the informed consents. Patients with acute exacerbation, intra and extrapulmonary infection, acute and chronic kidney disorder, diabetes mellitus, cardiac disease, neuromusculoskeletal abnormality, intra and extrapulmonary malignancy, active smoker, uric acid level of < 4 mg/dl, previously had allopurinol consumption, antioxidant consumption such as vitamin C and E, diuretic, cyclophosphamide, probenecid, anticoagulant at least a week prior to the start of this study, and post thorax or abdominal surgery were excluded from this study.

The study subjects were grouped into intervention (n=19) and control (n=19) groups at the beginning of the study, however, one subject in the control group experienced traffic accident and could not continue this study so at the end of the study there were only 37 subjects. Allopurinol 300mg/day single dose was given to the intervention group for 4 weeks (28 days) while the control group received nothing but their standard therapy for COPD.

The data comprised of categorical and numerical data. Chi-square or Fisher exact test was used to analyzing categorical data. Numerical data was presented in mean with standard deviation. All data were statistically assessed with SPSS version 17 and p-value of < 0.05 was considered significant.

Adverse events of allopurinol were monitored by phone calls and direct examinations during their routine follow-ups at the outpatient clinic. None of the subjects reported any adverse event during this study period. This study had been approved by research ethics committee of Dr. Moewardi hospital/Faculty of Medicine of Universitas Sebelas Maret, Surakarta.

3. Results

Baseline characteristics of the subjects were age, gender, education, occupation, body weight, body height, body mass index (BMI), smoking status, yearly exacerbations, and COPD groups. Subjects characteristics' data and study variables of both groups were statistically homogenous with a p-value > 0.005 as could be seen in Table 1.

3.1. Glutathione Sulfhydryl (GSH) serum level

Before the intervention, the GSH level of the intervention group was 97.80±25.64 µg/ml while the control group was 99.19±32.23 µg/ml. By the end of the study, the GSH level of intervention group decreased significantly into 45.22±23.52 µg/ml (p=0.000). Meanwhile, the control group which received standard therapy alone experienced a slight decrease (89.85 ± 35.84 µg/ml; p=0,291). The difference of GSH level (post-pre) between the two groups was statistically significant (p=0.001). The GSH serum level results were shown in Table 2.

3.2. Percentage of forced expiratory volume in 1 second (%FEV_1)

The value of %FEV_1 at baseline in both intervention and control groups were 40.34±17.40 and 38.66±15.41 respectively. After four weeks the value decreased significantly, they became 33.42±16.89 and 30.23±11.67 in intervention and control groups respectively. However, when both groups were compared statistically, no significant difference was found (p=0.940). Table 3 showed the value of %FEV_1 in this study.

3.3. Six-minute walking test (6MWT)

The 6MWT results of both groups intervention and control at baseline were 255.05±67.23 m and 227.72±38.42 m, respectively. By the end of the study period, the intervention group had a significant improvement in 6 MWT (265.00±62.08 m; p=0.005). There was also an improvement in the control group. However, it was not statistically significant (233.33±38.56 m; p=0.109). Statistically, there was no difference in 6MWT between the two groups (p=0.057). The 6MWT results were showed in Table 4.

Table 1: Baseline Characteristics of the Subjects.

Variables	Groups		
	Intervention (n = 19)	Control (n = 18)	p-value
Gender[3]			
Male	16(84.2%)	15(83.3%)	0.942
Female	3(15.8%)	3(16.7%)	
Age[1]	65.32±5.99	65.83±7.51	0.968
Educations[3]			
No education	1(5.3%)	0(0.0%)	0.479
Elementary	10(52.6%)	7(38.9%)	
Junior high school	3(15.8%)	3(16.7%)	
Senior high school/STM	5(26.3%)	5(27.8%)	
Diploma 2	0(0.0%)	2(11.1%)	
Diploma 3	0(0.0%)	1(5.6%)	
Occupations[3]			
Army/Police	2(10.5%)	1(5.6%)	
Government officer	2(10.5%)	2(11.1%)	
Teacher	0(0.0%)	1(5.6%)	
Private officer	6(31.6%)	2(11.1%)	
Labor	2(10.5%)	6(33.3%)	0.484
Farmer	2(10.5%)	2(11.1%)	
House-wife	3(15.8%)	2(11.1%)	
Security	0(0.0%)	1(5.6%)	
Midwife	0(0.0%)	1(5.6%)	
Village officer	1(5.3%)	0(0.0%)	
Becak driver	1(5.3%)	0(0.0%)	
Weight[1]	52.16±11.35	53.39±11.34	0.065
Height[1]	159.42±6.35	161.56±4.69	0.32
BMI[1]	20.60±4.81	20.53±4.59	0.132
Uric acid level[1]	5.48±1.11	5.21±1.41	0.642
Smoking Status[3]			
Smoker			
Mild Brinkman Index (BI)	1(5.3%)	0(0.0%)	0.537
Moderate BI	9(47.4%)	6(33.3%)	
Severe BI	6(31.6%)	9(50%)	
Non-smoker	3(15.8%)	3(16.7%)	
COPD Groups[3]			
C	3(15.8%)	5(27.8%)	0.314
D	16(84.2%)	13(72.2%)	
Yearly exacerbations[2]	1.58±0.69	1.50±0.79	0.62

Notes: [1]independent sample T-test, [2]Mann Whitney, and [3]Chi square

Table 2: Glutathione Sulfhydryl (GSH) Serum Level Pre, Post, and The Difference Between Intervention and Control Groups.

Groups	Glutathione Sulfhydryl Serum Level			
	Pre	Post	p	Δ (Post-Pre)
Intervention	97.80 ± 25.64	45.22 ± 23.52	0.000[1]	-52.58 ± 38.39
Control	99.19 ± 32.23	89.85 ± 35.84	0.291[1]	-9.34 ± 36.35
p	0.558[2]	0.000[2]		0.001[2]
Notes: [1]paired sample t-test, [2]Mann Whitney.				

Table 3: Percentage of Forced Expiratory Volume in 1 second (%FEV_1) Pre, Post, and The Difference Between Intervention and Control Groups.

Groups	Percentage of Forced Expiratory Volume in 1 second			
	Pre	Post	p	Δ (Post-Pre)
Intervention	40.34 ± 17.40	33.42 ± 16.89	0.011[1]	6.91 ± 10.60
Control	38.66 ± 15.41	30.23 ± 11.67	0.006[1]	8.43 ± 11.26
p	0.759[2]	0.775[3]		0.940[3]
Notes: [1]paired sample t-test,[2]independent sample t-test, [3] Mann Whitney.				

Table 4: Six-minute walking test (6MWT) Pre, Post, and The Difference Between Intervention and Control Groups.

Groups	Six-minute walking test			
	Pre	Post	p	Δ (Post-Pre)
Intervention	255.05 ± 67.23	265.00 ± 62.08	0.005[1]	9.95 ± 13.73
Control	227.72±38.42	233.33±38.56	0.109[1]	5.61 ± 14.08
p	0.141[2]	0.073[2]		0.057[3]
Notes: [1]paired sample t-test,[2]independent sample t-test, [3]Mann Whitney.				

3.4. COPD assessment test (CAT) score

The mean CAT score of intervention and control groups at entry point were 22.57±7.60 and 18.67±7.38, respectively. In the intervention group, the score decreased significantly after the administration of allopurinol (12.16±4.63; p=0.000). There was no significant decrease in CAT score in the control group at the end of the study period (17.50±7.70; p=0.085). A statistically significant difference was observed in both groups (p=0.000). Table 5 showed the results of CAT score in this study.

4. Discussion

Allopurinol had been proved to play a role as antioxidative stress which is assessed by the changing of GSH serum level while its role as anti-inflammation could not be proved in this study as the value of %FEV_1 remained decreased. Males were affected

TABLE 5: COPD assessment test (CAT) Score Pre, Post, and The Difference Between Intervention and Control Groups.

Groups	COPD assessment test Score			
	Pre	Post	p	Δ (Post-Pre)
Intervention	22.53 ± 7.60	12.16 ± 4.63	0.000[1]	-10.37 ± 4.46
Control	18.67 ± 7.38	17.50 ± 7.70	0.085[1]	-1.17 ± 2.71
p	0.118[2]	0.014[3]		0.000[2]

Notes: [1]paired sample t, [2]Mann Whitney, [3]independent sample t-test.

by COPD more than females were in this study. Education and occupation may also influence COPD occurrence. Low education is related to poor knowledge on the dangers of smoking habit, cigarette smoke, and exposure to noxious agents which affect health, particularly in the lungs. The majority of study subjects in the intervention group were employees while in the control group were labors. Global initiative for COPD declares that low socioeconomy is one of the COPD risk factors, presumably, it is related to in an outdoor pollutant exposure, the crowd, poor nutrition, infection and other socioeconomic related factors [1, 3, 14]. Eighty percent of our study subjects were smokers. Cigarette smoke exposure was classified based on BI, and most of the subjects were categorized as moderate. High smoking habit is one of the factors affecting high COPD incidence [3]. Despite the baseline characteristics differences between the two groups, the two groups were homogenous statistically so the variables could be compared one another.

Glutathione sulfhydryl originates in liver and functions as endogenous antioxidant found in sulfhydryl cysteine binding. Organs which mainly use GSH production in the liver as an antioxidant are lungs, kidney, and gut [15]. Our findings revealed that the GSH level in the allopurinol group decreased significantly. Allopurinol 300mg given daily as a single dose for four weeks lowered GSH level. This was due to oxidative stress regulator effect owned by allopurinol. This finding was similar to Ichinose et al. and Heunks et al. findings. Ichinose et al. reported that allopurinol 300mg daily as XO inhibitor given for four weeks suppressed the production of peroxynitrite and RNS, while Heunks et al. found that allopurinol 300mg daily administered for two days lowered the formation of GSSG and MDA [9, 10, 12].

COPD patients experience decreased FEV_1 progressively every year by 50 to 60 ml [16, 17]. As in our study the $\%FEV_1$ value decreased in both groups. Ichinose et al. also reported that there was no change in respiratory tract volume of subjects given allopurinol 300mg/day [12]. This phenomenon was due to persistent inflammation process

in the respiratory tract and lung parenchyma leading to progressive respiratory tract obstruction and finally caused airway remodeling in COPD patients.

COPD patients experience decreased exercise capacity because of reduced muscle mass, shortness of breath, and airway obstruction. The 6MWT is the most common method used in lung rehabilitation program since it is cheap, easy to use, and practical. It is also a reliable indicator. Thus we used it in this study. The minimal significant difference (MID) in 6MWT according to European Respiratory Society (ERS) dan American Thoracic Society (ATS) guideline is 30 m [18, 19]. The administration of allopurinol as antioxidant could reduce muscle damage which was expected to lessen skeletal muscle tissue damage, including diaphragm muscle of COPD patients. Our findings showed that COPD patients given allopurinol had significant improvement in their exercise capacity clinical symptoms of stable COPD patients was important to be evaluated. This finding could not be compared to other studies since it was the first study that ever investigated about it. Factors that could influence the 6MWT finding in this study were allopurinol effect as XO inhibitor, predominantly male gender in this study who had high motivation in doing 6MWT, and most of the subjects had ever done 6MWT before [20].

Worsened clinical symptoms can detain the daily activity of COPD patients resulting in a decrease of QoL. CAT is used to measure the QoL of COPD patients. This study used score showing improved clinical symptoms was a decrease of 2-3 points [1, 21, 22]. In our findings allopurinol affected on decreasing the CAT score of stable COPD patients group C and D. However this study could not be compared to others as this was the first study whichever investigated the effect of allopurinol on clinical symptoms of stable COPD group C and D. Allopurinol 300 mg daily inhibited XO enzyme and prevented the formation of free radicals so that oxidative stress process could be prevented systematically, thus skeletal injury was impeded resulting in improved respiratory muscle contractility and skeletal muscle. Therefore shortness of breath and peripheral skeletal muscle dysfunction ameliorated which eventually improved clinical symptoms as well as exercise capacity [9, 10, 12]. As a limitation, this study was only able to prove the hypothesis of allopurinol effects as an antioxidant on GSH serum level, 6MWT, and CAT score of stable COPD group C and D while effect as anti-inflammation had not been proved. Therefore a further study was needed to prove the anti-inflammatory effect of allopurinol.

5. Conclusions

Allopurinol 300 mg daily given for 4 weeks significantly lowered GSH serum level, improved 6MWT, and reduced CAT score but did not affect the %FEV_1 value of patients with stable COPD group C and D.

References

[1] GOLD 2017 Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease *Global Initiative for Chronic Obstructive Lung Disease Inc*. **2017** 1-123

[2] Senior RM, Pierce RA, Atkinson J 2015 Chronic obstructive pulmonary disease: epidemiology, pathophysiology, pathogenesis, and α1-antitrypsin deficiency *Fishman's pulmonary diseases and disorders*. Vol 5, ed Grippi MA, Elias JA, Fishman JA, Kotloff RM, Pack AI, Senior R (New York: McGraw-Hill Education) chapter 40 pp 613-45

[3] Amin M, Yunus F, Antariksa B, Djajalaksana S, Wiyono W 2016 *PPOK diagnosis dan penatalaksanaan*. Vol 1 (Jakarta: Penerbit Universitas Indonesia) p 1-111

[4] Barnes PJ 2013 New anti-inflammatory targets for chronic obstructive pulmonary disease *Nat Rev Drug Discov*. **12** 543-59

[5] Rahman I 2012 Pharmacological antioxidant strategies as therapeutic interventions for COPD *Biochim Biophys Acta*. **1822** 714-28

[6] Pirabbasi E and Cheragi M. 2012 Antioxidant supplementation among COPD: is it necessary? *Pakistan J Nutr*. **11** 501-6

[7] Kirkham PA and Barnes PJ. 2013 Oxidative stress in COPD *Chest*. **144** 266-73

[8] Pouwels SD, Heijink IH, Hacken NHT, Vandenabeele P, Krysko DV, Nawijn MC, van Oosterhout AJM 2014 DAMPs activating innate and adaptive immune responses in COPD *Mucosal Immunology.* **7** 1-12

[9] Heunks LMA and Dekhuijzen PNR 2000 Respiratory muscle function and free radicals: from cell to COPD *Thorax*. **55** 704-16

[10] Ryan MJ, Jackson JR, Hao Y, Leonard SS, Alway SE 2011 Inhibition of xanthine oxidase reduces oxidative stress and improves skeletal muscle function in response to electrically stimulated isometric contractions in aged mice *Free Radic Biol Med*. **51** 38-52

[11] Macnee W, Vestbo J, Agusti A 2016 COPD: pathogenesis and natural history *Murray & Nadel's textbook of respiratory medicine*. Vol 6,ed Broaddus VC, Mason RJ, Ernst

JD, King TE, Lazarus RC, Murray JF, Nadel JA, Slutsky AS (Philadelphia: Elsevier Saunders) chapter 43 pp 751-89

[12] Ichinose M, Sugiura H, Yamagata S, Koarai A, Tomaki M, Ogawa H, Komaki Y, Barnes PJ, Shirato K, Hattori T 2003 Xanthine oxidase inhibition reduces reactive nitrogen species production in COPD airways *Eur Respir J*. **22** 457-61

[13] Raharjo A 2017 *Thesis Pengaruh latihan harmonika pada kapasitas inspirasi, gejala sesak napas, kapasitas latihan, dan kualitas hidup penderita PPOK* (Surakarta: Departement of Pulmonology and Respiratory Medicine Medical Faculty: Universitas Sebelas Maret)

[14] Widyastuti K 2017 *Thesis Pengaruh exercise berjalan berbasis pedometer dan standar terhadap aktivitas fisik, gejala sesak napas, kapasitas exercise, dan kualitas hidup penderita PPOK stabil*. (Surakarta: Departement of Pulmonology and Respiratory Medicine Medical Faculty: Universitas Sebelas Maret)

[15] Morris D, Khurasany M, Nguyen T, Kim J, Guilford F, Mehta R, Gray D, Saviola B, Venketaraman V 2013 Glutathione and infection *Biochim Biophys Acta - Gen Subj*. **1830** 3329-49

[16] Hyatt R 2014 Spirometry: dynamic lung volumes *Interpretation of pulmonary function test: a practical guide*. Vol 4, ed Hyatt RE, Scanlon PD, Nakamura M (Philadelphia: Walters Kluwer Health) chapter 2 pp. 4-22

[17] Hellebrandová L, Chlumský J, Vostatek P, Novák D, Rýznarová Z, Bunc V 2016 Airflow limitation is accompanied by diaphragm dysfunction *Physiol Res.* **65** 469-79

[18] Rochester CL, Vogiatzis I, Holland AE, Lareau SC, Marciniuk DD, Spruit M 2015 An official American Thoracic Society/European Respiratory Society policy statement: enhancing implementation, use, and delivery of pulmonary rehabilitation *Am J Respir Crit Care Med* **192** 1373-86

[19] Holland AE, et al 2014 An official European respiratory society/American thoracic society technical standard: field walking tests in chronic respiratory disease *Eur Respir J*. **44** 1428-46

[20] Enright P 2003 The Six-Minute Walk Test *Respir Car*e. **48** 783-5

[21] Karloh M, Mayer AF, Maurici R, Pizzichini MMM, Jones PW, Pizzichini E 2016 The COPD assessment test: What do we know so far? A systematic review and meta-analysis about clinical outcomes prediction and classification of patients into gold stages *Chest*. **149** 413-425

[22] Smid DE, Franssen FME, Houben-Wilke S, Vanfleteren LEGW, Janssen DJA, Wouters EFM, Spruit MA 2017 Responsiveness and MCID estimates for CAT, CCQ, and HADS

in patients with COPD undergoing pulmonary rehabilitation: a prospective analysis *J Am Med Dir Assoc*. **18** 53-80