Available online at www.sciencedirect.com

ScienceDirect

Procedia Chemistry 18 (2016) 194 – 198

Procedia Chemistry

Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 Conference, MCLS 2015

The Correlation between Pulmonary Function Tests and The Salivary MMP-9 Activity among Chronic Obstructive Pulmonary Disease (COPD) Patients

Mulyadi[a]*, Sunnati[b], Mulkan Azhary[c]

[a]*Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Syiah Kuala University, Kopelma Darussalam, Banda Aceh 23111, Indonesia*

[b]*Department of Periodontics, Faculty of Dentistry, Syiah Kuala University, Kopelma Darussalam, Banda Aceh 23111, Indonesia*

[c]*Department of Anatomy and Histology, Faculty of Medicine, Syiah Kuala University, Kopelma Darussalam, Banda Aceh 23111, Indonesia*

Abstract

The spirometry test is routinely performed to assess FEV_1, FVC, and FEV_1/FVC ratio among chronic obstructive pulmonary disease (COPD) patients with the increased activity of MMP-9. Saliva is less invasive to assess the MMP-9 activity. This study aimed to compare Pulmonary Function Tests to estimate the MMP-9 activity. The respondents were 30 COPD outpatients from Pulmonary Policlinic. Results showed mean ratio of FEV_1, FVC, FEV_1/FVC (SD) and that of the salivary MMP-9 activity were 1.67 (0.12) L, 2.97 (0.43) L, 56.15 (8.43) % and 1.85 (1.54) μM respectively. The correlation between FEV_1, FVC, and FEV_1/FVC ratio and the salivary MMP-9 activity was insignificant (p>0.05). The pulmonary function tests were not able to estimate the salivary MMP-9 activity. The findings suggest further activities of MMP-9 from other samples for comparison of protease activity.

Peer-review under responsibility of the organizing committee of the Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 (MCLS 2015)

Keywords: Spirometry; Salivary; MMP-9; Comparison

* Corresponding author. Tel.:. +62-813-3557-1574
E-mail address: mul.0862@gmail.com

Peer-review under responsibility of the organizing committee of the Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 (MCLS 2015)
doi:10.1016/j.proche.2016.01.030

Nomenclature

BAL	Broncho-alveolar lavage
COPD	Chronic obstructive pulmonary disease
ECM	Extracellular matrix
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GOLD	Global initiative for chronic obstructive lung disease
MMP-9	Matrix metalloproteinase-9
TIMP-1	Tissue inhibitor of metalloproteinase-1

1. Introduction

The prevalence of chronic obstructive pulmonary disease (COPD) in Indonesia has reached 4.8 millions of which 90% is smokers or former smokers. COPD is mainly triggered by smoke exposures and other dangerous agents like gases and chemical substances from the environment. The genetic inheritance, previous history of respiratory infection, intrauterine growth retardation (IUGR), poor nutrition, and low income also contribute to the increase of COPD. The passive smokers, approximately 20%, are also risky for getting COPD[1,2].

Spirometry is routinely performed by those complaining dyspnoe promptly, so that they may have the appropriate treatment subsequently. The calibrated spirometry is initially prepared before patients start the test. Then, the measurements of forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) are performed with acceptable maneuvers. The results are very beneficial to determine either obstruction, restriction, or mixed. Regarding the diagnosis of COPD, the baseline of GOLD 2013 is properly applied[1,2,3].

The increase of matrix metalloproteinase (MMP)-9 activity from plasma is correlated with α_1-anti tripsin-related emphysema[4,5]. Moreover, the higher MMP-9 level is linear with the lower FEV_1, transport of CO, and oxygen saturation. The increase of MMP-9 level might estimate the decline of pulmonary function and the risk of exacerbation. The cigarette consumption is also correlated with MMP-9 level[4]. Prior authors and colleagues have started numerous studies to determine the correlation between different biological biomarkers and the pulmonary function. For COPD, many biomarkers from broncho-alveolar lavage (BAL) fluid and sputum have been developed in order to generate precise relationships between types of biomarkers and pulmonary function. Types of biomarkers engaged in those studies were agents of oxidative stress, cytokines and various proteases describing the pathogenesis of COPD. However, how to get the respiratory samples should be considered as well as those body fluids which have not been easily obtained[5,6,7,8].

Since smoking would affect the pathological processes of oral cavity and lungs which induced inflammatory mediators releasing MMP-9, our study tried to utilize the saliva to assess the MMP-9 activity from the fluid in order to link the association between spirometry results and the activity of protease. Furthermore, hopefully, the spirometry tests might be correlated adequately to the salivary MMP-9 activity among COPD patients.

2. Methods

This study employed thirty (30) smoker outpatients with COPD who visited pulmonary policlinic of Dr. Zainoel Abidin General Hospital in Banda Aceh - Indonesia. The study applied cross sectional design to determine inclusion and exclusion criteria for those enrolled as respondents. In advance, they were confirmed as COPD patients based on spirometry results i.e. FEV_1/FVC ratio <70% with mild, moderate, severe and very severe GOLD spirometric level[3]. Other criteria were: smokers, male, >50 years old, 20 pack years of cigarette consumption. The exclusion criteria were tuberculosis and malignancy. The study was officially approved by the Ethics Committee of Faculty of Medicine, Syiah Kuala University - Banda Aceh. All respondents had to sign the informed consents before undergoing the physical examination and saliva collection.

The saliva was collected by spitting out the fluid into the sterile pot. Respondents who had shown the worst oral hygiene and bloody spittle discharge were excluded from the study. All collected saliva was stored in -80 ^{0}C freezer for subsequent analysis with *Sensolyte®520 Generic MMP assay kit Fluorimetric*.

3. Results and discussion

3.1. Characteristics of respondents' spirometry test and salivary MMP-9 activity

This research employed 30 respondents who were diagnosed with COPD regardless GOLD obstruction criteria. The spirometry tests showed mean ratio of FEV_1, FVC, and FEV_1/FVC (SD) i.e. 1.67 (0.12) L, 2.97 (0.43) L and 56.15 (8.43) % respectively. Moreover, the salivary MMP-9 activity was 1.85 (1.54) μM. Spirometry results showed that FEV_1/FVC ratio was <70% confirming that respondents suffered from COPD. Data were shown in Table 1.

Table 1. Characteristics of respondent's spirometry test and salivary MMP-9 activity

Variables	n	Mean (SD)
FEV_1 (L)	30	1.67 (0.12)
FVC (L)	30	2.97 (0.43)
FEV_1/FVC ratio (%)	30	56.15 (8.43)
Salivary MMP-9 activity (μM)	30	1.85 (1.54)

3.2. Correlation between FEV_1, FVC, FEV_1/FVC ratio and the salivary MMP-9 activity

The salivary MMP-9 activity was correlated with spirometry tests by using Pearson correlation test. All spirometry values showed lower correlation towards the salivary MMP-9 activity with insignificant *p* values ($p>0.05$) as shown in Table 2. The pulmonary function tests might not conclude predicted enzyme activity of MMP-9.

Table 2. Correlation of FEV_1, FVC, and FEV_1/FVC ratio towards salivary MMP-9 activity

	Salivary MMP-9 activity	
	r	*p* value
FEV_1 (L)	0.119	0.532
FVC (L)	0.018	0.923
FEV_1/FVC ratio (%)	0.161	0.396

3.3. Discussion

Smokers with COPD underwent a decrease in FEV_1 and FVC, faster than non smokers, and so did the age accelerating the decrease concurrently. FEV_1/FVC ratio is one of the considerations to initially recognize the obstructive diseases to determine whether COPD-suspected patients should be treated as suffering from obstructive lung disease or not. The estimated value of FEV_1/FVC ratio should be confirmed and determined more appropriately[3,4,9]. Our study revealed spirometry results accordingly to diagnose COPD accurately (Table 1).

Inflammatory progression on respiratory tracts, induced by smoking exposure, led to the release of numerous inflammatory cells predominantly neutrophils and macrophages that subsequently generated MMP-9. Release of MMP-9 was also led by some cytokines and oxidative stress. Beside increased level of MMP-9, the balance between MMP-9 and tissue inhibitor of metalloproteinase (TIMP)-1 also influenced the degradation since TIMP-1 was the main anti protease maintaining the balance with MMP-9 in order to prevent action of active MMP-9. According to

spirometry values, the decline of FEV_1 was linear with obstruction of airway caused by breakage of lung extracellular matrix (ECM)[4,10].

From periodontal tissue, periodontitis had more activity of protease, particularly MMP-9, released by neutrophils and fibroblasts together with some cytokines e.g. IL-6 and IL-10. Similar to what occured in lung tissues, increased activity of MMP-9 and MMP-9/TIMP-1 ratio would destruct extracellular matrix which finally caused periodontitis[11,12,13].

This study analyzed the salivary MMP-9 activity as the saliva was easily collected with a simple procedure. Previous studies have assessed the level and activity of MMP-9 from various body fluids, mostly sputum, bronchoalveolar lavage (BAL) and blood serum[5,6,7]. Increased activity of MMP-9 may cleave ECM of lung tissues. Normally, those suffering from COPD, with lower FEV_1 and FEV_1/FVC ratio <70%, would also exhibit the increased level and activity of MMP-9. However, the activity assessment was more appropriate as such activity might represent the breakage of ECM than might the level of MMP-9. Instead of both pro MMP-9 and bond MMP-9, the activity only measured active MMP-9[14].

The tide of MMP-9 activity apparently showed a dynamic sequence. Healthy smokers would exhibit MMP-9 activity which was insignificant as compared to smokers with COPD. Prolonged smoking exposure was supposed to be responsible upon MMP-9 activity. Moreover, TIMP-1 also appeared to maintain the balance. Increased activity of MMP-9 corresponded with the airway obstruction and the destruction of the extracellular matrix among COPD patients. Moreover, the airway obstruction occured along with the increase of MMP-9/TIMP-1 ratio[9].

Spirometry also displayed dynamic values of pulmonary function test among COPD patients and so did MMP-9 activity. Our study was initialized with determining correlation between spirometry value and MMP-9 activity. Unfortunately, FEV_1, FVC, and FEV_1/FVC ratio correlated poorly and was insignificant (p>0.05) with salivary MMP-9 activity (Table 2). The protease activity of MMP-9 apparently had its individual characteristics despite its dynamical sequence. Smoke, mostly from cigarettes, entered through the oral cavity. This smoke, then, went into lungs, yet the saliva was apparent to correspond better to the processes of the oral cavity diseases[12,13]. The genetic factor was supposed to affect the variability of MMP-9 activity among COPD respondents. The genetic susceptibility would be responsible to express response of MMP-9 activity towards exposures, particularly smoking exposure. In addition, role of TIMP-1 also facilitated the feedback toward MMP-9 activity[15].

Due to inadequately comparable results of spirometry values towards salivary MMP-9 activity, this study proposed to discover other MMP-9 activities from related biological samples. In this regards, we might suggest further evaluation on MMP-9 activity from sputum (preferably) and BAL. Then, various activities of MMP-9 would be compared to each other. The results would be valuable to estimate degradation of ECM from lung parenchyma among COPD patients derived from some associated biological fluids[5,6,7].

4. Conclusions

Since FEV_1, FVC, and FEV_1/FVC ratio showed insignificant p value towards the salivary MMP-9 activity (p>0.05), pulmonary function tests apparently were poorly correlated with the salivary MMP-9 activity despite the similar exposure, i.e. smoking. In the future, similar studies should deepen included criteria for those suitably gained from many phenotypes of COPD patients in order to assess and estimate destruction of lung parenchyma caused by increased activity of MMP-9 in accord with spirometry's FEV_1, FVC, and FEV_1/FVC ratio.

Acknowledgements

We are very grateful to the Indonesian Ministry of Research-Technology and Higher Education and Syiah Kuala University for 2015 Fundamental Research Grant Batch 1.

References

1. Anwar D, Chan Y, Basyar M. Hubungan Derajat Sesak Napas Penderita Penyakit Paru Obstruktif Kronik Menurut Kuosioner Modified Medical Research Council Scale dengan Derajat Penyakit Paru Obstruktif Kronik. *J Respir Indo* 2012;32(4):200-207.
2. Wijaya O, Sartono TR, Djajalaksana S, Maharani A J. Peningkatan Persentase Makrofag dan Neutrofil pada Sputum Penderita Penyakit Paru Obstruktif Kronik Berhubungan dengan Tingginya Skor COPD Assessment Test (CAT). *J Respir Indo* 2012;32(4):240-249.
3. *Global Strategy for the Diagnosis, Management and Prevention of COPD*, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015. Available from: http://www.goldcopd.org/.

4. Omachi TA, Eisner MD, Rames A, Markovtsova L, Blanc PD. Matrix Metalloproteinase-9 Predicts Pulmonary Status Declines in α_1-Antitrypsin Deficiency. *Respiratory Research* 2011;12:35:1-11.
5. Cazzola M, Novelli G. Biomarkers in COPD. *Pulmonary Pharmacology & Therapeutics* 2010;23(6):493-500.
6. Barnes PJ, Chowdhury B, Kharitonov SA, Magnussen H, Page CP, Postma D, Saetta M. Pulmonary Biomarkers in Chronic Obstructive Pulmonary Disease, *Am J Respir Crit Care Med* 2006;Vol 174:6-14.
7. Snell N, Newbold P. The Clinical Utility of Biomarkers in Asthma and COPD. *Current Opinion in Pharmacology* 2008;8(3):222-235.
8. Yigla M, Berkovich Y, Nagler RM. Oxidative Stress Indices in COPD-Broncho-alveolar Lavage and Salivary Analysis. *Archives of Oral Biology* 2007;52(1):36-43.
9. Abdella AM, Atti GA, Eed MA, Eldib AS, Haleem SS. Evaluation of matrix metalloprotease-9 and tissue inhibitor metalloprotease-1 levels in bronchoalveolar lavage of apparently healthy smokers. *Egypt. J. Chest Dis. Tuberc* 2015, http://dx.doi.org/10.1016/j.ejcdt.2014.12.001.
10. Kang MJ, Oh Y-M, Lee JC, Kim DG, Park MJ, Lee MG, Hyun IG, Han SK, Shim Y-S, Jung K-S. Lung Matrix Metalloprotease-9 Correlates with Cigarette Smoking and Obstruction of Airflow. *J Korean Med Sci* 2003;18:821-7.
11. Verstappen J, Von den Hoff JW. Tissue Inhibitors of Metalloproteinases (TIMPs): Their Biological Functions and Involvement in Oral diseases, *J Dent Res* 2006;85:1074.
12. Rai B, Kharb S, Jain R, Anand SC. Biomarkers of Periodontitis in Oral Fluid. *Journal of Oral Science* 2008;50(1):53-56.
13. Rai B, Kaur J, Jain R, Anand SC. Levels of gingival crevicular metalloproteinases-8 and -9 in periodontitis. *Saudi Dent J* 2010;22:129-131.
14. Lowrey GE, Henderson N, Blakey JD, Corne JM, Johnson SR. MMP-9 protein level does not reflect overall MMP activity in the airway of patients with COPD. *Resp Med* 2008;**102:**845-851.
15. Fujita M. The Role of MMPs in the Progression of Chronic Lung Inflammatory Diseases, In: Ong K-C, (Editor). *Lung Inflammation*, ISBN: 978-953-51-1373-7, InTech; 2014 DOI: 10.5772/57391. Available from: http://www.intechopen.com/books/lung-inflammation/the-role-of-mmps-in-the-progression-of-chronic-lung-inflammatory-diseases.