The Role of Alpha-1 Antitrypsin Enzyme on Pulmonary Dysfunction Due to Exposure to NO$_2$

[1]Satria Pratama, [1]Bambang Wispriyono, [1]Haryoto Kusnoputranto and [2]Faisal Yunus
[1]Department of Environmental Health, Faculty of Public Health,
[2]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine,
Universitas Indonesia, Depok, Indonesia

Abstract: Nitrogen dioxide (NO$_2$) is one of the air pollutants whose concentration is increasing due to the increased number of vehicles as a mode of transportation. Exposure to NO$_2$ causes physical symptoms of respiratory disorders and lung function and Alpha 1 Antitrypsin (AAT) plays a role in the protection of inflammation due to exposure to NO$_2$ through inactivation by nitrogen. This study aims to reveal the relationship of NO$_2$ exposure, AAT and lung function of the traffic police in Jakarta. It is a cross sectional study with 112 traffic police as research subjects divided into two groups: the police working on the highway and working in the office. NO$_2$ measurement of the environment is carried out at the location where the subjects work and examination is done toward lung physiology, AAT levels, body weight, body fat percentage, height and waist circumference as a factor in determining lung function. NO$_2$ levels are 18.72 ppb on highways (high exposure areas) and 4.14 ppb in the office (low exposure area), respectively. There are 49% of the traffic police working on the highway (high exposure to NO$_2$) and 51% of police officers working in the office (low exposure to NO$_2$). Mean of pulmonary physiology, represented by percentage of FVC (Forced Vital Capacity), on the entire subject is 89.49 and mean of FEV$_1$ (Forced Expiratory Volume in 1 sec) is 95.39. The mean of FEV$_1$ compared to FVC is 88.4 and there is no significant difference between police officers working in the highway and working in the office. AAT levels in both subjects show normal values compared to normal levels of AAT standard of 141.07 mg/dL. Finally, the prediction model obtained in the end which is the prediction of FVC percentage and FEV$_1$ percentage for both the dependent variable has a normal distribution. In conclusion, the value of lung function for police officers working on the roads is lower than the value of lung function of those working in the office. Factors that affect lung function in this study are NO$_2$ levels, AAT, fat, age (35 years old), visceral fat and waist circumference.

Key words: NO$_2$, AAT, FEV$_1$, FVC, traffic police, Jakarta, Indonesia

INTRODUCTION

Air pollution can occur indoors (indoor pollution) and outside (outdoor pollution). Outdoor pollution is generally caused by pollution from motor vehicle fumes in dustrial, marine and natural processes of living beings. Sources of air pollutants can be classified into stationary sources and mobile sources and those can develop of COPD (Ko and Hui, 2012). Nitrogen dioxide (NO$_2$), Sulfur dioxide (SO$_2$) and Carbon monoxide (CO) are examples of air pollutant dangerous at room temperature. NO$_2$ exposure levels were found to be significantly associated with distance of residence from a main road and vehicle utilization (Hong *et al.*, 2005). High intensity exposure to NO$_2$ in a confined space could result in serious injury including death. Exposure to NO$_2$ at room temperature increases the risk of respiratory infections through the interaction of pollutants with the immune system

(Chen *et al.*, 2007). High concentrations of NO$_2$ in urban areas with high population of motor vehicles will cause or aggravate asthma, cough, respiratory diseases reactive airway inflammation and chronic respiratory disease (Fields, 2004) and genetic association studies have been the most feasible study design for investigating the interactions between genetics and the adverse effects of air pollutants in humans (Yang *et al.*, 2008).

Nitrogen dioxide is an air pollutant that is influential in generating emphysematous like lesions in the lungs of animals. Despite pathogenesis processes that generate these lesions is still unclear, the mechanism includes the possibility similar to the occurrence of emphysema in smokers. The occurrence of emphysema is due to degradation of the alveoli rich in elastin as the result of uncontrolled proteolysis due to endogenous elastase. Enzymes α1-proteinase inhibitor have a major role in controlling the activity of neutrophil elastase

Corresponding Author: Bambang Wispriyono, Department of Environmental Health, Faculty of Public Health,
Universitas Indonesia, Depok, Indonesia

(Johnson *et al.*, 1990). Mohsenin and Gee are also conducting studies to measure the activity of the enzyme α1-proteinase inhibitor obtained from BAL fluid (Broncho Alveolar Lavage) on the subject of non-smokers exposed to NO_2 for 3 h at a dose of 3-4 ppm and then do the bronchial rinse 3-4 h after exposure to NO_2. The results of this study found NO_2 causes 45% reduction of enzyme activity of α1-proteinase inhibitor. This decrease makes neutrophil elastase destroy elastin in the alveoli (Mohsenin and Gee, 1987). α1-proteinase inhibitor are also plays important role in smoking induced emphysema (Evans and Pryor, 1994).

Alpha 1 Antitrypsin (AAT) could prove to be an important marker protein in the exacerbation of COPD (Koczulla *et al.*, 2012) and there is significant associations between spirometric decline rate acceleration and AAT deficiency severity in FDNY workforce during the first 4 years after WTC-related inhalation injury (Banauch *et al.*, 2010). AAT deficiency is closely related to the occurrence of pulmonary emphysema appearing at the age of 35-45 years is chronic as progressive fatigue and other non-specific symptoms. AAT deficiency is often associated with the PI ZZ genotype and very rarely with the PI SZ genotype. In addition, to AAT deficiency, patients with PI ZZ genotype are patients with other genotypes such as PI MZ and PI MM. Patients with Chronic Obstructive Pulmonary Disease (COPD) with the PI MZ genotype experience a decrease in lung function faster than COPD patients with the PI MM genotype (Serres and Blanco, 2014). Sorheim *et al.* (2010) conduct a case study (COPD Patients post BD FEV_1/FVC <0.7 and FEV_1 <80% predicted) with the number of 834 patients and control (Patients with normal spirometer) with the number of 835 patients. The result is that PI MZ is the predictor for the low ratio of FEV_1/FVC. Based on multivariate analysis, PI MZ has a ratio of FEV_1/FVC 3.5% lower compared to PI MM; PI MZ is more susceptible to the development of airway obstruction interference than PI MM (Sorheim *et al.*, 2010).

The purpose of this study is to determine the levels of AAT, NO_2 levels of workplace air and pulmonary function impairment and determine factors most influential to lung function of traffic police. This study covers the environment NO_2 levels, AAT levels and value of lung function (Forced vital capacity and forced expiratory volume in 1 sec) on the traffic police in Jakarta.

MATERIALS AND METHODS

Based on the research objectives, the research uses cross sectional study design. Cross sectional assess exposure and the status of a disease or disorder as an outcome simultaneously at one time. This design is used for research that is not reported routinely and requires

measurements to identify cases. Data on the incidence of health is the prevalence or the proportion of people in a population affected by the outcome (Heederik and Teschke, 2005). Epidemiological research environment put a cross sectional view to determining the sample with high exposure group and compared with external comparators (low exposure group) (Baker, 2008).

The study assesses NO_2 levels, AAT levels and the environment as the main independent variable. All of these variables will be assessed simultaneously at one time to assess the magnitude of the problems that exist in the population. Therefore, the cross-sectional design is the most appropriate design to answer the purposes of this study. The study is conducted on a specific group of community with regard to exposure where the selection of the study population is carried out by exposure status police officers working on the roads or highways as a group of high exposure and those working in the office as the external comparison. The study uses a calculation of different sample mean and obtains 52 samples for each group and the total sample of 112. At the time of the sampling in terview, the measurement of height, weight, body fat, visceral fat, body mass index and spirometry as well as blood sampling for checking levels of the enzyme AAT is done.

RESULTS AND DISCUSSION

Different characteristics of the subject between the low exposure group and high exposure group covers average levels of NO_2, gender, age, height, weight, BMI (Body Mass Index), waist circumference, fat, visceral fat, levels of AAT can be seen in Table 1. The results of the independent variable characteristics between the two groups all have non-significant difference except the fat variables and levels of the enzyme of AAT.

This study obtain spirometry test results to determine lung function as dependent variables between low exposure and high exposure group as shown in

Table 1: Characteristics of the independent variables between low exposure group and high exposure group

Variables	Exposed	Low exposed	p-values
NO_2 levels (ppb)	18.72	4.14	0.398
Sex (N(%))	-	-	0.000
Male-	54 (100)	43 (76.8%)	-
Female	0 (0%)	13 (23.2%)	-
Age (average (year))	37.55 (8.58)	39.01 (9.47)	0.399
Height (cm)	169.4	169	0.678
Weight (kg)	81.0	81.9	0.714
Body nass (kg/m²) index	28.2	28.6	0.618
Waist (cm) circumference	96.6	93.5	0.202
Fat (%)	25.9	28.7	0.007
Visceral fat	14.0	13.6	0.688
Alpha 1 anti trypsin (mg/dL)	151.9	130.6	0.002

Table 2: Different lung functions between low exposure group and high exposure group

Dependent variable (%)	High exposure	Low exposure	p-values	K-S test
FVC	88.6	90.2	0.449	0.20
FEV_1	94.5	96.1	0.500	0.20
FEV_1/FVC	87.8	88.9	0.416	0.00
FEF2575	115.6	115.0	0.905	0.20
APE	69.1	79.2	0.006	0.02

Table 3: Correlation of the dependent variable with normal distribution toward variables

Independent variables	Dependent variable	
	FEV_1 (%)	FCV (%)
Age		
r	-0.006	-0.182
P	0.951	0.057
Fat		
r	-0.217	-0.286
P	0.023	0.002
BMI		
r	-0.209	-0.303
P	0.029	0.001
Visceral fat		
r	-0.178	-0.315
P	0.063	0.001

Table 2. Pulmonary function tests between the 2 groups result in non-significant different except for the variable Peak Expiratory Flow (PEF). In Table 2, it can also be seen the value of Kolmogorov-Smirnov (K-S test) to see the data distribution of the dependent variable. K-S test on variables of FEV_1, FVC, FEF 2575 (Forced Expiratory Flow) shows a normal distribution.

Correlation test on the dependent variable with normal distribution of lung function shows that only age variable has a non-significant correlation (Table 3). Overall, the independent variables have negative correlation with lung function seen with R-values that all are negative. Figure 1 shows a scatter plot graph on the correlation of independent variables with normal distribution on lung function. Variables percentage of fat and body mass index although, significant shows a weak correlation against the percentage of FEV_1 (-0.217 and -0.209) and the percentage of FCV (-0.286 and -0.303). Variable visceral fat has a significant correlation but the correlation is very weak against the percentage of FEV_1 (-0.178) and FCV (-0.315).

Multivariate analysis performed in this study aims to look at the most decisive factor of lung function for police officers working on highways. The primary independent variable is the concentration of NO_2 that represents the condition of two locations: in the office and the environment. The other variable as priority is AAT. Variable AAT has a curve sloping to the left and the results of Kolmogorov-Smirnov test is p = 0.00, thus, modification by changing the AAT into 1/AAT variable. The 1/AAT variable has a curve sloping to the right of the

Kolmogorov-Smirnov test and p = 0.002. The dependent variable used of lung function is the percentage of FCV and FEV_1, both have a normal distribution in the normality test so they can be analyzed using linear regression method.

The first stage is to find the regression of the dependent variable FEV_1. Variable of NO_2 levels is still included in the linear regression test because the levels of NO_2 is the most important independent variable and 1/AAT variable is still included as a major independent variable because it is part of the research hypothesis. The independent variable of age is replaced with categorical variables of age groups on dichotomous nominal variables such as age >35 years old and <35 years old. The result of the analysis is:

$$FEV_1 \, (\%) = 126.643 - 0.198 \times NO_2 - 179.824 \times (1/AAT)$$
$$-0.413 \times (fat) - 0.76 \times (age \, 35) + 0.204 \times$$
$$(VF) - 0.196 \times (LP)$$

Discussions include data collection, laboratory analysis, relationship of NO_2 exposure with some of the factors that will affect lung function and limitations of the study. Problems that arise are all police officers (whether working in the office or on the highways) still have an obligation to work on the highways at least twice a month, so, it will be difficult to find research subjects that have never served in the traffic. Sampling of the ambient NO_2 is carried out according to rush hour traffic to get results of maximum NO_2 levels. Blood sampling for AAT test does not experience problems because blood taken is the blood serum, stored in the refrigerator so it does not lyse. The results of all blood samples could be examined. Two subjects are excluded because after examination and interview, one had a history of chronic diseases and one is a new officer, so, the total subjects who successfully enrolled in the study are110 people with a composition of 56 people in the office and 54 subjects on highways.

The examination on the level of ambient NO_2 is done using Griess Saltzman method with a spectrophotometer. The results show that NO_2 levels are higher on highways than in the office with a value of 18.72 and 4.14 ppb. Although, the environmental data obtained seem high, this data is far below the threshold value set by Jakarta Province's Environment Agency (BPLHD) at 400 ppb. Indonesia's threshold value is the same as the threshold value set in Europe (Sandström, 1995). The threshold value for the measurement of 1 hour in Indonesia is much higher than that set by the US to the region of US, i.e, . 200 ppb (Anonymous, 2008).
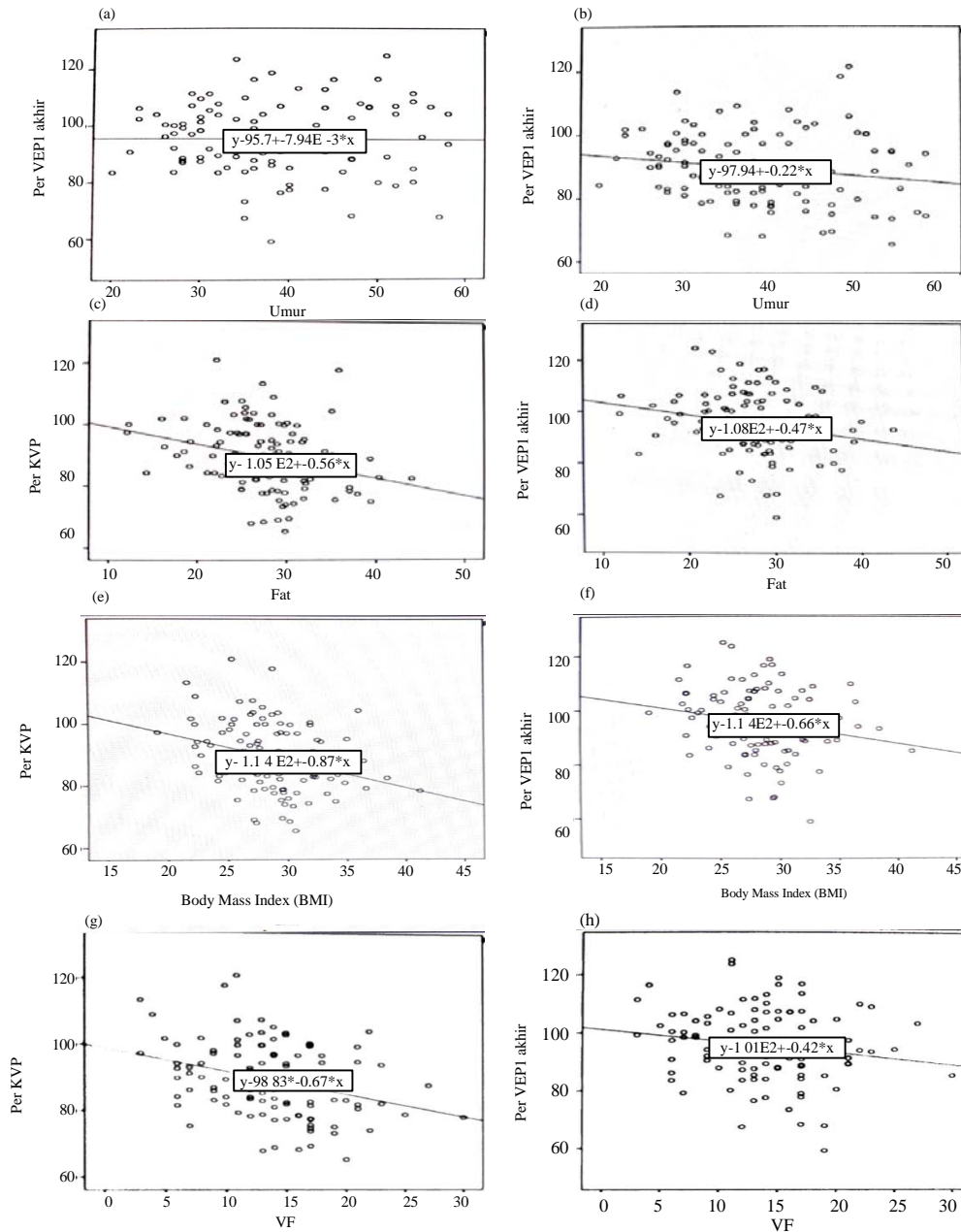
Fig. 1: a) R, linear-3519E-S; b) R, linear-0033; c) R, linear-0.082; d) R, linear-0.047; e) R, linear-0.092; f) R, linear-0.044; g) R, linear-0.099; h) R, linear-0.032; A scatter plot graph on the correlation of independent variables (age, fat, body mass index and visceral fat) with normal distribution on lung function

Forced Vital Capacity (FVC) is a value to determine disruptions of lung restriction. FVC value is a parameter to determine pulmonary function to know abnormalities in restriction or lung development. This value is also used as a comparison of the value of Forced Expiratory Volume in 1 sec (FEV$_1$) to determine airway obstruction (Dermawan and Yunus, 2013). Forced vital capacity percent value in this study shows no significant difference between subjects exposed to high exposure and low exposure even though the average value for the high exposure group is lower than the low exposure group 88.68 and 90.27. These results differ from those by Prashant Patil and others who conduct research on traffic police and police officers working in an office that there is significant difference between the high exposure group and the low exposure group at 77 and 88.8 (Patil *et al.*,

2013). The different results of forced vital capacity between the two groups in the present study could occur because the mean for variables theoretically associated with forced vital capacity values such as height, weight, waist circumference and age do not differ significantly between the two groups.

FEV_1 is important to know how much obstruction (respiratory disorders) that occurs. The results of the research show a mean value of FEV_1 percentage between groups exposed to high and low exposure is not significant although the mean value of FEV_1 percentage in the high exposure group is lower. FEV_1 value is quite important because the symptoms are often directly from gas exposure on the respiratory tract. Results obtained if categorized based on the obstruction disorder by dividing the normal value of FEV_1 percentage >80% and FEV_1 percentage <80% among high exposure and low exposure group is 5 (9.2 %) and 6 (10.7 %) person. The two groups based on the test results are not significantly different.

There are significant differences among subjects exposed to high and low exposure to the levels of alpha 1 anti-trypsin. The mean levels of alpha 1 anti-trypsin on the subjects of high exposure have higher values 151.91 mg/dL than subjects exposed to low exposure 130.62. Although, there are differences in the mean levels of alpha 1 anti trypsin, both are still within the normal limit of more than 80 µg/mL. Examination using ELISA to determine levels of alpha 1 anti-trypsin as a diagnostic uses normal limit value of 80 mg/dL (Mulgrew *et al.*, 2007). The determination of this standard is different from the study conducted by Amin. Amin conducts a study on patients and employees of a factory as many as 418 subjects with the lowest levels determined 180mg/dL Amin, 2001). Johnson *et al.* (1990) conduct a study in healthy people exposed to NO_2 and control group (without NO_2) for 3 h in a random test double blind on the subjects and then continues with bronchoalveolar rinse with bronchoscopy to measure the activity of the enzyme alpha 1 proteinase inhibitor (alpha 1 antitrypsin) on liquids of bronchoalveolar lavage. The study shows strong evidence in the inactivation of inhaled AAT in low levels of NO_2 in the short term although it still needs further studies to determine the inhibition of AAT in NO_2 repeated exposure (Johnson *et al.*, 1990). The unique susceptibility of AAT-deficient individuals to exposure to chemicals and particulate environmental agents (De Serres, 2003) may also affected the result of AAT level in this study.

Determining factors that are most instrumental in lung function in this study is done using multivariate analysis without neglecting the essence of variables. After the selection of candidates and co-linearity, candidates will be tested in multivariate analyses. The results in this study show that multivariate test cannot be done ideally to get the parsimony model. The results of multivariate analysis in determining the value of FEV_1 percentage show that for every increase of 1 value of NO_2 will affect 19.8% of FEV_1. The explanation also applies in assessing the other variables in the model. These results such in studies by Park *et al.* (2012) assessing factors of body composition on lung function in 291 healthy subjects with fat percentage are the most significant factor in determining the impairment of FEV_1, although, in conclusion Park *et al.* (2012) state that predictions based on factors of body composition is not quite comprehensive in explaining the condition and to replace the standard lung function prediction formula today.

Monitoring biological processes is one of the three preventive devices of diseases caused by toxic materials and working environment in general as well as two other matters related to the environment and health monitoring or surveillance. The third stage of the presence of ambient concentrations of pollutants in air, water and food will finally reach the fourth stage, i.e., the dose of contaminants that enter the human body. The second stage and third stage can be done with the dynamics and mechanisms of transport. The third and fourth stage can be done by looking at the pattern of past exposure on human beings. The emergence of effect stage is undesirable health disorders. It depends on the dose response. Our previous study also showed one malondialdehyde as one of stress oxidative marker in pulmonary function of chromium's exposed workers (Wispriyono *et al.*, 2016). These studies have reached stage 3 and it still takes two more stages to determine whether exposure to NO_2 have an adverse effect on health, so, it is difficult to conclude a single causality of NO_2 as factors causing disruption of lung function on police officers working on highways. Endogenous NO could also influence the mechanism. NO endogenous can be increased by regulating the proinfalamtory mediators and AAT play a role in inhibiting cell activation and suppressing cytokine production which associated with proinflamatory stimulation (Chan *et al.*, 2012).

CONCLUSION

The ambient NO_2 levels obtained in this study on highways (high exposure areas) is higher compare with in the office (low exposure area), although, it is not significantly different. However, the value of lung function for police officers working on the roads is lower than the value of lung function of those working in the office and the levels of AAT is statistically significant. The factors affecting lung function of FEV_1 percentage for officers working on highways based are the levels of NO_2, AAT, visceral fat and waist circumference.

ACKNOWLEDGEMENTS

REFERENCES

Amin, M., 2001. The role of alpha-1-antitrypsin in generating chronic obstructive pulmonary disorder. Respirology, 6: S39-S43.

Anonymous, 2008. Risk and exposure assesment to support the review of the NO2 primary national ambient air quality standard. Environment Protection Department, United States Environmental Protection Agency, Washington, D.C., USA. https://www3.epa.gov/ttn/naaqs/standards/nox/dat a/20081121_NO2_REA_final.pdf

Banauch, G.I., M. Brantly, G. Izbicki, C. Hall and A. Shanske *et al.*, 2010. Accelerated spirometric decline in New York City firefighters with a1-antitrypsin deficiency. Chest J., 138: 1116-1124.

Chan, E.D., G.B. Pott, P.E. Silkoff, A.H. Ralston and C.L. Bryan *et al.*, 2012. Alpha-1-antitrypsin inhibits nitric oxide production. J. Leukocyte Boil., 92: 1251-1260.

Chen, T.M., W.G. Kuschner, J. Gokhale and S. Shofer, 2007. Outdoor air pollution: Nitrogen dioxide, sulfur dioxide and carbon monoxide health effects. Am. J. Med. Sci., 333: 249-256.

De Serres, F.J., 2003. Alpha-1 antitrypsin deficiency is not a rare disease but a disease that is rarely diagnosed. Environ. Health Perspect., 111: 1851-1854.

Dermawan, R. and F. Yunus, 2013. [Fixed rate diagnostic test against normal lower limit VEP1/KVP for assessing breath channel obstruction (In Indonesian)]. J. Respir. Indo., 33: 210-220.

Evans, M.D. and W.A. Pryor, 1994. Cigarette smoking, emphysema and damage to alpha1-proteinase inhibitor. Am. J. Physiol. Lung Cell Mol. Physiol., 266: L593-L611.

Fields, S., 2004. Global nitrogen: Cycling out of control. Environ. Health Perspect., 112: A556-A563.

Heederick, D. and K. Teschke, 2005. Exposure Assessment for Pesticides in Epidemiological Studies. In: Occupational and Residential Exposure Assessment for Pesticides, Franklin, C.A. J.P. Worgan (Eds.). John Wiley & Sons, Hoboken, New Jersey, USA., pp: 245-274.

Hong, Y.C., J.H. Leem, K.H. Lee, D.H. Park and J.Y. Jang *et al.*, 2005. Exposure to air pollution and pulmonary function in university students. Intl. Arch. Occup. Environ. Health, 78: 132-138.

Johnson, D.A., M.W. Frampton, R.S. Winters, P.E. Morrow and M.J. Utell, 1990. Inhalation of nitrogen dioxide fails to reduce the activity of human lung alpha-1-proteinase inhibitor. Am. Rev. Respir. Dis., 142: 758-762.

Ko, F.W. and D.S. Hui, 2012. Air pollution and chronic obstructive pulmonary disease. Respirology, 17: 395-401.

Koczulla, A.R., S. Noeske, C. Herr, J. Koepke and R.A. Jorres et al., 2012. Alpha-1 antitrypsin is elevated in exhaled breath condensate and serum in exacerbated COPD patients. Respir. Med., 106: 120-126.

Mohsenin, V. and J.B.L. Gee, 1987. Acute effect of nitrogen dioxide exposure on the functional activity of alpha-1-protease inhibitor in bronchoalveolar lavage fluid of normal subjects. Am. J. Respir. Crit. Care Med., 136: 646-650.

Mulgrew, A.T., C.C. Taggart and N.G. McElvaney, 2007. Alpha-1-antitrypsin deficiency: Current concepts. Lung, 185: 191-201.

Park, J.E., J.H. Chung, K.H. Lee and K.C. Shin, 2012. The effect of body composition on pulmonary function. Tuberculosis Respire. Dis., 72: 433-440.

Patil, P.J., G.V. Thakaer and S.P. Patil, 2013. Comparative study of lung function test of policemen in traffic control with those in general duty. Natl. J. Physiol. Pharm. Pharmacol., 3: 162-166.

Sandstrom, T., 1995. Respiratory effects of air pollutants: Experimental studies in humans. Eur. Respir. J., 8: 976-995.

Serres, F.D. and I. Blanco, 2014. Role of alpha-1 antitrypsin in human health and disease. J. Internal Med., 276: 311-335.

Sorheim, I.C., P. Bakke, A. Gulsvik, S.G. Pillai and A. Johannessen *et al.*, 2010. a1-Antitrypsin protease inhibitor MZ heterozygosity is associated with airflow obstruction in two large cohorts. Chest J., 138: 1125-1132.

Wispriyono, B., R. Kurniawan, R. Novirsa, B. Hartono and M. Sari, 2016. Pulmonary function and Malondialdehyde (MDA) content in blood due to chromium exposure among tannery workers in Sukaregang, Garut. Res. J. Environ. Toxicol., 10: 183-188.

Yang, I.A., K.M. Fong, P.V. Zimmerman, S.T. Holgate and J.W. Holloway, 2008. Genetic susceptibility to the respiratory effects of air pollution. Thorax, 63: 555-563.