International Journal of scientific research and management (IJSRM)
\||Volume||3||Issue||7||Pages|| 3296-3300||2015||
Website: www.ijsrm.in ISSN (e): 2321-3418

Increasing The TNF-α Levels In Nasal Lavage Liquid And Pulmonary Function Decrease Of Rice Mill Operator Exposed To Lipopolysaccharide (LPS) Endotoxin

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Abstract: *Background: Exposure to endotoxin lipopolysaccharide in rice dust causes the inflammationinrespiratory tractwhich is characterized by an increase in Tumor Necrosis Factor Alpha (TNF-α) as well as the decline in pulmonary function of the rice mill operators. Objective: The aim of this study was to analyze the correlated of endotoxin LPS to increased levels of TNF-α in nasal lavage liquid and pulmonary function decline of the rice mill operators. Methods: This study was designed as a prospective longitudinal study.Dust sampling conducted during 8 hourswhile sampling the nasal lavage fluid and pulmonary function measurements made before and after the work (cross shift). Levels of TNF-α and endotoxin LPS subsequently analyzed using ELISA method, afterwards LPS analyzed by LAL method. Pulmonary function was analyzed by spirometry. Results: The mean levels of endotoxin LPS in the dust of ricewas 56.36±5.83 EU/m3. There are enhancement in the levels of TNF-α after the work (paired-samples t test:p=0.000) in which TNF-α levels increased in all rice mill operator after work. FVC decline of 72.73% and 63.64% for FEV1. Pulmonary function status after the work is: normal =36.36%, obstruction and restriction =36.36% =27.27%. Level of LPS endotoxin correlated to the increase in TNF-α in nasal lavage fluid workers (multiple linear regression test:p =0.000). Endotoxin LPS correlated tothe value ΔFVC and ΔFEV1 (multiple linear regression test:p <0.05). Conclusion: Endotoxin lipopolysaccharide is a factor affecting the increase in TNF-α level in nasal lavage fluid and pulmonary function decline rice mill operator. Suggestion: Please use a rice Mill machine equipped with a dust collector. Need periodic medical examination and treatment and rest or rolling work for workers who experience respiratory problems. Workers are advised to always using masks while working.*

Keywords: LPS endotoxin, pulmonary function, TNF-α, nasal lavage, rice mill operator**.**

1. INTRODUCTION

Occupational disease commonly experienced by those of work in the agricultural sector is a respiratory disease or disorder. Dust is a major risk factor that causes the respiratory disease or disorder. International Labour Organization (ILO) reported that approximately 40.000 new cases of pneumoconiosis (respiratory diseases due to dust) occur worldwide each year (Antarudin, 2003).

Rice Mill industry generate high dust and highly at risk to workers. Exposured to rice dust containing endotoxin lipopolysaccharide (LPS) may cause inflammationofrespiratory tract (Poole, 2012). Local inflammation occurs 4 to 6 hours after exposure. LPS inflammatory response in the form of phagocytosis by macrophages that produce proinflammatory cytokines Interleukin-1, Interleukin-6 and Tumor Necrosis Factor Alpha (TNF-α). Furthermore, TNF-α-induced stimulation of circulating neutrophils to migrate to the inflamed tissue to perform phagocytosis to endotoxin LPS (O'Gradi et al., 2001; Herman, 2006: Schwartz et al., 1992).

Inflammation causes excessive mucus production and acute respiratory obstruction which can recover (reversible), unless the exposure to long-term or persistent, it can cause chronic respiratory disorders and is irreversible such as chronic bronchitis and pulmonary fibrosis which causes a decrease in pulmonary function (Kennedy et al., 1994; Djojodibroto, 2014).

Studies that have been conducted by Rahardjo (2010) shows the results showed no relationship between the amount of dust with decreased pulmonary function of worker (p =0.003). Putranto (2007) in his research suggests that the dust concentration 229 mg/m³ led to a decline in pulmonary function as much as 31% of workers between the ages of 20 to 45 years. Furthermore, other studies on dust grains show the occurrence of inflammation that is characterized by the release of specific mediators of inflammation such as macrophages, neutrophils, and airway epithelial cells (Becker et al., 1999). Furthermore, other studies conducted in experimental animals using corn dust extract showed the presence of inflammatory response shown by the increase in total neutrophils (p =0.001) and the levels of TNF-α (p =0.04) in the liquid Bronchoalveolar Lavage (BAL) (Clapp et al., 1994). Research on rice dust exposure have shown a relationship between LPS with an inflammatory response characterized by proinflammatory cytokines such as Interleuki-1 (IL-1), Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)

on examination blood serum rice mill workers (Antarudin 2003; Sirait, 2010; Gezondheidsraad, 2011).

In general, conducted a study of inflammatory markers in serum and BAL media, while research by analyzing markers of inflammation in nasal lavage fluid (rinsing the nose) is still very limited. therefore the purpose of this study was toanalyzing biomarkers of acute local inflammation in this case is TNF-α in nasal lavage fluid as well as its correlation with a decrease in pulmonary function of rice mill operators are exposed to endotoxin LPS.

2. RESEARCH METHODS

Acute inflammation caused by LPS endotoxin occurs within 4 to 6 hours after exposure, therefore, the design of this study is a longitudinal study in which sampling and inspection is done before and after the work (cross shift) on 11 rice mill operator and performed on the first day of work after two days off. Dust concentration measurement using personal dust sampler (PDS) for 8 hours of work and analysis of the dust concentration with gavimetri method. Measurement of LPS endotoxin levels in dust personal conducted using Limulus AmoebocyteLysate (LAL) -Test.

The sampling procedure Nasal Lavage (NL) refers to Keman et al (1998) as follows: Provide instructions carefully to respondents on the objectives and procedures of nasal lavage fluid sampling and confirmed its respondents understand. Respondents in a sitting position and was directed to lift the neck up to 450, opened his mouth as wide-width to raise the ceiling to close nasophatring and holding their breath during sampling. A total of 10 ml of sterile phosphate buffered saline solution (0.9%) NaCl put into a sterile pipette measuring 10 ml. furthermore inserted in to each nostril respondents 5 ml phosphate buffered saline solution. After 10 seconds of respondents lowered his head to remove fluid into the centrifuge tube measuring 15 ml which has arranged sterile gauze to filter out the mucus. If the time before the laboratory examination of more than 4 hours, the samples are stored in containers that contained ice. Then the sample in a centrifuge at 600 g for 10 minutes. Supernatant is divided into eight aliquos (500 mL) stored at -70 0C for his next analyzed.

Examination of TNF-α was performed using indirect ELISA (Sandwich) with Human TNF-α reagent immunoassay from R & D Systems. It refers to the complete procedure (Indahwati, 2013). Examination of pulmonary function with a spirometer performed with the parameters FVC and FEV1.

Data were analyzed using statistical program. Test data normality using Shapiro Wilk test sample is less than 50. Paired samples t test for know the differences in levels of TNF-α and pulmonary function before and after work (cross shift). Linear regression was used to analyze the influence of the independent variable levels of dust, endotoxin LPS and worker characteristics (age, years of service and smoking habits) on the dependent variable that is increased levels of TNF-α in nasal lavage fluid rice mill worker and the pulmonary function rice mill worker

3. RESULTS AND DISCUSSION

1. Measurement results personal dust and LPS endotoxin levels

Personal dust levels are taken using personal dust sampler (PDS) with units of mg/m3.Endotkoksin levels of lipopolysaccharide in personal dust were analyzed using a sandwich ELISA technique. There is no threshold value for the concentration of endotoxin LPS. Distribution of personal dust and LPS endotoxin levels on respondents can be seen in the table below:

Table 1 Distribution of Personal Dust and LPS Endotoxin Levels in Rice Mill Operator Palur village, District Kebonsari, Madiun District in 2015.

Value	Rice Mill Dust Level	LPS Endotoxin Level
Minimum	1.25mg/m3	48EU/m3
Maximum	3.19 mg/m3	66EU/m3
Means±SD	2.199±0.723mg/m3	56.36±5.83 EU/m3
≤TLV	8 respondent	-
≥TLV	3 respondent	-

Table 1 shows 8 or 72.73% dust levels below the Threshold LimitValue (TLV) and only 3 or 27.27% which exceeds the specified TLV by The Ministry of Labor in Indonesia No. 3 of 2011 on TLV physical and chemical exposures in the workplace. The value of the lowest levels of personal dust was 1.25 mg / m3 and the highest levels of personal dust were 3.19 mg/m3. The average level of personal dust was2.199±0.723mg/m3. Variations personal dust content of each respondent is influenced by the presence of each respondent in the process of work where there are respondents who work in the rice mill with higher dust concentrations compared with workers in charge of the cleaning process and packaging of rice. Table 1 shows the lowest levels of endotoxin LPS was 48 EU/m3, while the highest level was 66 EU/m3. The average concentration of endotoxin LPS was 56.36±5.83 EU/m3.

2. Measurement Results Levels of Tumor Necrosis Factor Alpha Nasal Lavage Fluids

Intake of nasal lavage fluid samples for levels of TNF-α inspection carried out before and after work (cross shift). Afterwords, examination of the levels of TNF-α in nasal lavage fluid conducted in the laboratory using ELISA method. Statistical analysis of the results of laboratory examination of the levels of TNF-α is a descriptive and test differences in the levels of TNF-α before and after work.

Descriptive analysis of TNF-α levels which include the average number and standard deviation, minimum and maximum levels of TNF-α, as well as test results Samples Paired t test to see the difference in the levels of TNF-α in nasal lavage fluid rice mill operator before and after work can be seen in the table below:

Table 2 Distribution and Differences Before and After Working Levels of Tumor Necrosis Factor Alpha in Nasal Lavage Fluids Operator Rice Mill Palur village, Kebonsari subdistrict, Madiun district in 2015.

Analysis Indicator	TNF-α Levels (pg/ml)		
	Before Work	After work	Δ TNF-α
Minimum	0.00	11.50	3.00
Maximum	39.10	55.90	19.5
Means ± SD	13.27±11.90	23.98±12.11	10.68±5.25
Paired t-test	p=0.000**		

Note : **= significant

Table 2 presents the results of measurements of the levels of TNF-α before and after work where an increase in the levels of TNF-α after working on all respondents. TNF-α level are lowest before and after the work is 0 pg / mL and 11.50 pg / ml. The highest levels before and after the work was 39.10 pg / ml and after work 55.90 pg / ml. The average levels of TNF-α before the work was 13.27 pg / ml and after work was 23.98 pg / ml. Results Paired Samples t test showed no significant difference in highly TNF-α levels before and after working with p = 0.000.

3. Measurement Results Pulmonary Physiology Respondents Before and After Work

Results of pulmonary function tests with parameters FVC and FEV1 values showed no difference before and after work. Her complete as can be seen in the following table:

Table 3 Percentage Change Value FVC and FEV1 Before and After Working on Rice Mill Workers at Palur village, KebonsariSubdistrict, MadiunDistrict in 2015.

	Δ FVC		Δ FEV1	
	n	%	n	%
Increase	3	27,27	4	36,36
Decrease	8	72,73	7	63,64
Totaly	11	100	11	100

Table 3 shows the results of pulmonary function where a decline in FVC after works amounting to 72.73%, while for the parameter FEV1 decreased by 63.64% of all workers were examined.

4. Results of pulmonary function status identification rice mill operator

Pulmonary function is checked using a spirometer with FVC and FEV1 indicators in order to obtain the status of pulmonary obstruction, restriction and a mixture of obstruction and restriction which is called the mix. The following shows the status of pulmonary rice mill operator.

Table 4 Distribution Workers Pulmonary Faal Satus Rice Mill Palur village, Kebonsari Subdistrict, Madiun District in 2015.

Status of Pulmonary Function	Before Work		After work	
	n	%	n	%
Normaly	6	54.54	4	36,36
Obstruction	4	36,36	4	36,36
Restriction	1	0.09	3	27,27
Mixed	-	-	-	-
Totaly	11	100	11	100

Table 4 shows values above normal pulmonary function before work is in addition to the 54.54% 27.28% obstructed and 0:09% experienced restriction, whereas after the work is known that normal pulmonary function at 36.36%, 36.36% and 27 obstruction, 27% were experiencing restrictions.

5. Effect of rice mill dust and lipopolysaccharide endotoxin levels to increase of TNF-αlevels and decrease of pulmonary function of The Rice Mill Operators

Increase of TNF-α levels in nasal lavage fluid and descreaseof pulmonary function of the rice mill operator is influenced by personal dust and LPS endotoxin levels. Results of multiple linear regression test on the effect of levels of dust and endotoxin LPS can be seen in the following table:

Table 5 Effect of Rice Mill Dust and LPSEndotoxinLevels toIncreased of TNF-α levelsand Decrease of Pulmonary Function of The Rice MillOperator on PalurVillage, Kebonsarisubdistrict, Madiun district in 2015.

Independent Variabels	TNF-α	Pulmonary Function	
		Δ FVC	Δ FEV1
Personal Dust Levels	0.496	0.054	0.188
LPS Endotoxin Levels	0.000**	0.001**	0.033*

Note : * = Significant (p <0.05)
** = Very significant (p <0.01)

Table 5 shows the results of multiple linear regression test where endotoxin LPS effect on increase levels of TNF (p =0.000) and correlated to the decline in the value ofΔFVC (p =0.001) and ΔFEV1 (p =0.033).While the other variables that dust levels had no significant effect on the increased levels of TNF-α (multiple linear regression test: p>0.05).

4. DISCUSSION

These results indicate the average amount of dust that is 2:19 mg / m3 or still below the Threshold Limit Value (TLV) that is 3 mg / m3. Although still in bwah threshold value but if the dust exposure takes place continuously, it will result in a decrease in pulmonary function due to the accumulation of dust that accumulates in the airways to the alveoli (Sirait, 2010). Given also the average levels of endotoxin LPS in personal dust was 56.36 EU / m3. There is no limit on the levels of endotoxin LPS but some studies have found that levels of endotoxins is a risk factor for respiratory tract inflammation

occurs her (Rankin et al., 1996). The concentration of endotoxin LPS in the work environment is higher than the ambient air outside the work environment (Duquanne et al., 2012).

Results of the study also found elevated levels of TNF-α in all rice mill operators after work and test results Paired Samples t Test showed highly significant differences in the levels of TNF-α before and after work (cross shift). Elevated levels of TNF-α indicates the immune response due to exposure to dust containing rice Mill LPS endotoxin.

Endotosin LPS in the rice mills that inhaled dust will stick to the airway epithelial tissue and cause inflammation or inflammation characterized by signs such as redness, heat, pain and swelling in the airways. Furthermore, LPS endotoxin binds to its receptor which is lipopolysaccharide Binding Protein (LBP) so as to provide a stimulus for macrophages to perform phagocytosis. Besides macrophages will produceproinflammatory cytokines namely IL-1, IL-6 and TNF-α. Particularly proinflammatory cytokines TNF-α will stimulate spending for leukocyte adhesion molecules that are within the circulation selectin E and P selectin, ICAM and VICAM so leukocyte adhesion molecules will also issue selectin, LFA-1 and Mac-1, causing a weak attachment occurs between two molecules that leukocytes will feed stick to the endothelial cells/attachment will be stronger with the activation of chemotactic factors such as leukotiin, β4 and β6, PAF and IL-8 by increasing the affinity of leukocyte adhesion molecules for endothelial adhesion molecules. His next lei = ukosit in this case PMN (neutrophil) will penetrate the endothelial wall to the network and migrate to sites of inflammation to perform phagocytosis. Products of phagocytosis yakniu exudate that causes tissue to become swollen and leikosit dead will be an abscess or pus. This process will stop when all the dead LPS (Aggarwal., 2009; Baratawidjaja and Rengganis., 2013); Bastrad et al., 2006; Gioannini et al., 2003; Keman, 1997).

Dust levels do not affect the increase in TNF-α but endotoxin LPS in personal dust affect the increase in TNF-α. Meaning that the higher levels of endotoxin LPS then also increase the levels of TNF-α. It is the same with the results Leli (2013) who discovered the effect of endotoxin LPS to increased levels of TNF-α. Several other studies found results that there is an increase in pro-inflammatory cytokines such as TNF-α, IL-1, IL-6 and IL-8 as well as the number of cells of neutrophils and macrophages in the blood serum after exposure to endotoxin LPS in organic dust is Borm et al., (1996), Becker et al., (1999) and O'Gradi et al., (2001), Muller et al., (2006).

The results of measurements of pulmonary function rice Mill operators showed a decline in the value of FVC and FEV1 72.73% 63.63% while the status of normal pulmonary function before work is 6 respondents, 4 respondents obstruction and restriction one respondent. Normal pulmonary function status after working 4 4 respondents respondents obstruction and restriction 3 respondents. That is a change after working only experienced two respondents from normal to restriction.

Decrease in FVC and FEV1 values in pulmonary function measurements showed rice mill operator changes the air vent after work. It is influenced by endotoxin LPS according to the results of a study that found the effect of endotoxin LPS against ΔFVC highly significant ($p = 0.001$) and significantly to ΔFEV1 ($p = 0.033$). Inhaled endotoxin LPS which causes inflammation in the airways. Inflammation causes tissue damage of respiratory tract characterized by inflammation and excessive mucus secretion resulting in barriers to the flow of air in and out of the pulmonarys. Inflammation that causes the respiratory air flow resistance may be temporary and do not alter the structure and function of tissues and organs respiratory inflammation. If exposure to endotoxin LPS continues and the inflammation becomes chronic respiratory tissue and organ damage can be irreversible so that even chronic pulmonary function decline.

Wohlford et al., (1998) reported that exposure to organic dust from agricultural activities associated with the development respiratory tract diseases such as asthma and chronic airway obstruction that causes her obstructed airflow due to inflammation in the peripheral airways as a result of exposure to endotoxin lipopolysaccharide. Farida (2008) and Rianto (2009) also found an association between exposure to dust mills and a decrease in vital capacity pulmonarys workers

5. CONCLUSION

Increased levels of TNF-α in nasal lavage fluid after working rice mill operator. Elevated levels of TNF-α in nasal lavage fluid is influenced by lipopolysaccharide endotoxin in the dust inhaled by rice mill operator conducting dive activity. Endotoxin lipopolysaccharide to the decline in pulmonary function rice mill worker.

Therefore, it is necessary the use of rice Mill machines equipped with dust collector (dust collector) as well as the design of the workplace with proportional ventilation so that air circulation is better. There should be a periodic health examination so that workers can diagnose respiratory disorders to treatment and there is a policy to break or change the type of work that does not aggravate respiratory problems are at risk. It is also recommended for workers to always use the mask at the time of doing the job.

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