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Correlation of Lipopolysaccharide Endotoxin Level in Cotton Dust with the Increase of TNFα Level and the Decline of Lung Function in Cotton Spinning Factory Workers

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Abstract

Lipopolysaccharide (LPS) endotoxin contained in cotton dust may cause airway inflammation and decline of lung function when inhaled, which eventually leads to respiratory symptoms. The objective of this research is to analyze the correlation of the exposure of LPS endotoxin in cotton dust with the increase of TNFα level and the decline of lung function after one day's work. This study applied analytical observation method and prospective cohort approach. Main participants of this study were the workers of a cotton spinning factory located in Tulangan District, Sidoarjo Regency, East Java Province. Sixteen samples from cotton factory were taken as study group, and twenty three samples from village administrators were taken as control group. Data collection involves several techniques: spirometry, laboratory test, and interview. Results showed that concentration of personal dust has a significant relationship with the decline of FVC, %FVC, $FEV_{1,}$ and $\%FEV_1$, with Pearson correlation test showing $p<0.05$. LPS endotoxin in personal dust samples has a significant relationship with the increase of blood serum TNFα and the decline of FEV_1 and $\%FEV_1$, with Pearson correlation test showing $p>0.05$. The research concludes that the level of LPS endotoxin was strongly related to the increase of blood serum TNFα and the decline of lung function. Development of more effective preventive measures such as stronger enforcement of worker's health maintenance regulations and use of personal protective equipment is needed to ensure the best protection of cotton workers' health.

Abstrak

Korelasi Kadar Endotoksin LPS Dalam Debu Kapas dengan Peningkatan TNFα Serum Darah dan Penurunan Faal Paru Pekerja Pemintalan Kapas. Endotoksin LPS yang terkandung dalam debu kapas dapat menimbulkan reaksi inflamasi pada saluran pernafasan apabila terhirup yang selanjutnya dapat mempengaruhi faal paru dan menimbulkan gangguan pernafasan. Penelitian ini bertujuan untuk menganalisis hubungan paparan debu kapas dan endotoksin LPS didalamnya, dengan peningkatan kadar TNFα serum darah dan penurunan faal paru setelah bekerja dalam sehari. Penelitian ini merupakan penelitian analitik observasional dengan pendekatan kohor prospektif. Penelitian dilakukan pada pabrik pemintalan kapas di Kecamatan Tulangan, Sidoarjo dengan responden pekerja pemintalan yang bekerja pada shift pagi sebanyak 16 orang. Sebagai kelompok pembanding adalah perangkat Desa Singopadu dan Kajeksan, Kecamatan Tulangan. Pengumpulan data dilakukan dengan cara pemeriksaan spirometri, laboratorium dan wawancara. Hasil penelitian menunjukkan bahwa terdapat hubungan yang signifikan (korelasi Pearson, $p<0,05$) antara kadar debu kapas personal dengan penurunan FVC, %FVC, $cFEV_{1,}$ dan $\%FEV_1$. Kadar endotoksin LPS dalam debu kapas mempunyai hubungan yang signifikan terhadap peningkatan TNFα serum darah dan penurunan pada FEV_1 dan $\%FEV_1$ (korelasi Pearson, $p>0,05$). Untuk itu perlu adanya pelaksanaan program kesehatan dan keselamatan pekerja dengan benar oleh pemilik usaha untuk melindungi kesehatan pekerjanya.

Keywords: cotton dust, cotton workers, LPS endotoxin, lung function, serum TNFα

Introduction

Cotton spinning factory is a workplace with a great potential of causing occupational diseases in its workers because of frequent and continuous exposure to organic dust for approximately eight working hours each day. This intense exposure increases the risk for cotton factory workers of getting respiratory problems. A study

conducted by Prihata which examined the correlation between cotton dust concentration and lung function capacity of workers in the production division of a textile factory in Cirebon showed that the average concentration of cotton dust surrounding the main work area reached 1.584 mg/m^3, which decreased workers' lung function by 72.9%. This clearly increased the risk of developing various occupational diseases, especially respiratory problems. A syndrome called *byssinosis*—characterized by a feeling of pressure on the chest and symptoms of asphyxia on the first working day after a holiday—is frequently found affecting cotton factory workers. These symptoms gradually decrease when the workers leave the workplace. Such complaints are triggered by certain obstructions blocking their respiratory tracts.[1]

Dust inhaled by the workers triggers nonspecific natural defense mechanism, which manifests in coughing, sneezing, mucocilliary transport disorders, and phagocytosis by macrophages. Smooth muscle surrounding respiratory tract can inflame causing constriction. This condition usually occurs when dust concentration exceeds certain limits. Interaction with monocytes, macrophages, and neutrophils will release inflammatory mediators such as interleukin (IL), interferron (IFN), platelet-activating factor (PAF), and tumor necrosis factor alpha (TNFα).[2]

One of the agents contained in organic dust (including cotton dust) and triggering inflammation in human respiratory tract is the lipopolysaccharide (LPS) endotoxin. Endotoxin is a combination of lipid (lipid A) and polysaccharide chain in the outer membrane wall of negative gram bacteria, which is released to its surrounding environment during active cells development or during the lysis process or when the bacteria are phagocytized by macrophagic cells in human immune system. LPS endotoxin has been known to cause inflammation in exposed tissues, including lung tissues.

A study by Simpson et al. found that the concentration of cotton dust in the blowing division of a cotton factory reached 1.07 (0.72—5.9) mg/m^3 with endotoxin concentration of 9730 EU/m^3. This finding also demonstrated that cotton dust exposure can cause acute or chronic health impacts.[3,4]

In Indonesia, there have been many cases of occupational lung diseases or disorders which are caused by dust, especially cotton dust. This is due to the ever-increasing demand for cotton materials each year. However, research on the impacts of LPS endotoxin exposure borne by cotton dust on human respiratory tract is still rare.

This research was conducted in a cotton spinning factory located in Tulangan District, Sidoarjo Regency, East Java Province, with a production capacity of approximately two tons per day. This factory is chosen because of its relatively high concentration of cotton dust distributed all over the production area and the apparent unavailability of any dust collecting system, which certainly magnifies the potential for developing respiratory problems in its workers. An initial dust measurement showed a level of dust concentration exceeding the threshold limit value (*Nilai Ambang Batas* or NAB) as specified in the Minister of Labor and Transmigration's Regulation No. Per.13/Men/X/2011 on the Threshold Limit Value of Physical and Chemical Factors at Workplace; this threshold is set at 0.2 mg/m^3 for 8 working hours per day or for 40 working hours per week.[5]

The whole process of cotton spinning generates a large quantity of cotton dust, and the workers are always at high risk of being exposed to cotton dust containing LPS endotoxin. Frequent exposure of LPS endotoxin in cotton dust can increase the level of TNFα in the workers' blood serum, which triggers inflammatory reactions in their respiratory tracts, thus decreasing their lung function and generating various respiratory complaints. In addition to the level of internal dust (indoor), the level of personal dust (i.e. dust inhaled by individual worker) is a crucial factor contributing to the increase of TNFα level in the workers' blood serum and the decrease of lung function after working hours. A preliminary survey of potential health problems in the factory workers identified both minor and major complaints such as coughing, sputum, and asphyxia due to frequent inhalation of cotton dust at workplace. Besides being harmful to the workers' health, this hazardous condition also increases health care expenses and workers' absenteeism, which in turn reduce work productivity. Such condition calls for a regular examination of lung function for all cotton spinning factory workers.

The purpose of this research is to analyze the decrease of lung function in cotton spinning factory workers due to frequent exposure of LPS endotoxin in cotton dust.

Methods

This research is a non-intervention observational analytical project which applies prospective cohort study design. Samples were obtained from all sixteen morning shift workers at a cotton spinning factory (named CV "X") as study group and from administrative officers of Singopadu Village as control group. All respondents have met these inclusion criteria: (1) aged 18-60, (2) having no record of any chronic respiratory disease, (3) never working in other places with high level of dust exposure, and (4) willing to be research

respondents. Samples of control group were chosen using a simple random method, obtaining a total number of 23 unexposed respondents, all of whom are administrative officers of Singopadu and Kajeksen Villages, Tulangan District. Research was conducted from April to August 2013. Data were collected by (1) obtaining personal dust samples (using personal dust sampler) during working hours of the one shift, (2) measuring the workers' lung function using spirometer shortly before and after the working hours (8 hours) of the one shift, (3) taking the workers' blood samples, and (4) interviews. The research's bound variables were the increase of TNFα level in blood serum and the decrease of lung function after a day's work. TNFα level is measured by applying the sandwich ELISA technique with Human TNF alpha reagent kit.

The research's free variables were the level of personal dust measured gravimetrically and the level of LPS endotoxin in cotton dust measured using the Limulus Amebocyte Lysat (LAL) method. Gravimetry is a quantitative method of analysis which involves measuring the isolation process and the weight of a constituent. The first stage in gravimetry analysis is the separation of components to be examined from other components within the same sample; these separated components then undergo a sedimentation process, i.e. by transforming the constituents into a stable and pure compound which can be empirically measured. Gravimetry method is applied by measuring the weights of the components. LAL test is the most sensitive and specific method available for screening human medicine, biological medicine, animal medicine, medical implements, and raw materials for identifying potential levels of danger caused by the presence of endotoxin. LAL reagent is composed of blood cells of Atlantic horseshoe crab (*Limulus polyphemus*). *Limulus amebocyte Lysate* (LAL) is utilized to detect endotoxin related to negative gram bacteria.

Data analysis was conducted descriptively and analytically. Several tests were also applied in this research: (1) Shapiro Wilk data normality test with the number of samples <50, (2) paired sample difference test to examine the difference in lung function before and after working hours, and (3) free sample difference test to examine the differences in values between the exposed groups and the unexposed group. To determine the relationship between independent and dependent variables, the Pearson or Spearman correlation test was also employed.

Results and Discussion

This research involved cotton spinning workers with an average age of 42.13±10.43 and village administrative officers with an average age of 43.17±6.93. Table 1 shows that the average level of personal cotton dust in cotton factory workers is 7.26±1.89 mg/m^3 with the lowest value at 4.28 mg/m^3 and the highest value at 10.8 mg/m^3. In comparison, the average level of personal cotton dust in village administrators is 0.23±0.08 mg/m^3 with the lowest value at 0.102 mg/m^3 and the highest value at 0.351 mg/m^3.

Table 2 shows that the average levels of LPS endotoxin in personal cotton dust are 167.76±57.20 EU/m^3 in cotton factory workers and 1.37±4.05 EU/m^3 in village administrators.

As shown in Table 3, the result of the paired samples t test reveals a significant difference in cotton factory workers' lung function before and after working hours ($p<0.05$), with an exception of parameter %FEV1/FVC. On the other hand, despite a very slight change, village administrators' lung function does not show any significant difference before and after working hours.

Table 1. Distribution of Personal Dust Level Based on Workplaces, Year 2013

	Cotton Spinning Workers	Village Administrators
	Personal Dust Level (mg/m^3)	Personal Dust Level (mg/m^3)
Average ±SD	7.26±1.89	0.23±0,08
Min/max	4.28/10.80	0.10/0.35

Value presented in Average±SD; SD= Standar Deviasi

Table 2. Distribution of LPS Endotoxin Level in Personal Cotton Dust, Based on Workplaces, Year 2013

	LPS Endotoxin Level (EU/m^3)
Cotton Spinning Factory	167.76±57.20
Village Administration Offices	1.37±4.05
	p = 0.000

Value presented in Average±SD; LPS = Lipopolysaccharide

In order to find any difference in the TNFα level in workers' blood serum before and after working hours (cross shift), the paired sample t statistical test was administered. The comparison between the TNFα level in the workers blood serum and the TNFα level in the village administrators' blood serum is presented in Table 4. The test result shows a statistically significant difference of the TNFα level ($p<0.05$) in workers' blood serum before and after working hours in a day. In contrast, the TNFα level in village administrators' blood serum do not show any difference before and after working hours (cross shift).

As shown in Table 5, the result of the Pearson correlation test reveals a highly significant correlation of personal cotton dust level with the decrease in FVC, %FVC, FEV_1, and $\%FEV_1$ levels. In village administrators, no correlation of personal dust level with the increase of blood serum TNFα and the decrease of lung function is found. As shown in Table 6, the result of the Pearson correlation test shows a highly significant correlation (with $p<0.05$) of the level of LPS endotoxin in personal cotton dust with the increase of blood serum TNFα levell and the decrease of FEV_1 dan $\%FEV_1$ levels. These results demonstrate that the presence of LPS endotoxin in cotton dust is highly responsible for increasing the level of TNFα in human blood serum, which leads to the decrease of human lung function. It is clear that continuous exposure to cotton dust reduces workers' lung function after a day's work.

The size of the dust collected by the personal dust sampler is 5—10 micron, according to the device's specification. This kind of dust can be deposited anywhere within human upper respiratory tract. Small cotton dust particles are transported into the lung's alveoli by means of inhalation and are deposited there, damaging the alveoli and reducing their capacity to retain oxygen. Further accumulation of cotton dust will cause lung disorders and byssinosis.[6]

Table 3. Difference of Lung Function Value Before and After Working Hours (Cross Shift) Based on Workplaces, Year 2013

Parameter		Workplace	
		Cotton Spinning Factory	Village Administration Offices
FVC	Average±SD pre	2.302±0.309	2.789±0.519
	Average±SD post	2.09±0.365	2.78±0.615
	P	0.016	0.893
%FVC (%)	Average±SD pre	89.37±19.58	87.98±13.64
	Average±SD post	80.74±15.65	90.25±19.22
	P	0.022	0.444
FEV_1 (L)	Average±SD pre	2.917±0.758	2.482±0.447
	Average±SD post	2.538±0.536	2.415±0.502
	P	0.001	0.368
$\%FEV_1$ (%)	Average±SD pre	90.828±21.63	93.89±17.32
	Average±SD post	79.2±15.26	91.01±18.24
	P	0.002	0.301
$\%FEV_1$/FVC (%)	Average±SD pre	126.94±32.48	91.91±12.18
	Average±SD post	123.262±29.95	89.72±21.28
	P	0.480	0.554

Value represented in significance (p); FVC = Forced Vital Capacity

Table 4. Result of Respondents' Blood Serum TNFα Level Test Before and After Working Hours, Year 2013

Blood serum TNFα (pg/mL)	Workplace	
	Cotton Spinning Factory	Village Administration Offices
Average±SD (before work)	21.797±29.47	15.897±4.971
Average±SD (after work)	51.43±33.86	16.703±5.262
Average±SD increase of blood serum TNFα	29.63±19.76	0.805±1.77
Significance (p) of increase	0.000	0.427

Table 5. Correlation of Personal Dust Level with the Increase of Blood Serum TNFα Level and the Decrease of Lung Function, Year 2013

Variable	Cotton Spinning Workers		Village Administrators	
	Correlation Coefficient (r)	Significance (p)	Correlation Coefficient (r)	Significance (p)
Δ FVC	0.711	0.002	-0.109	0.620
Δ %FVC	0.667	0.005	-0.222	0.308
Δ FEV_1	0.653	0.006	0.244	0.255
Δ $\%FEV_1$	0.55	0.006	0.061	0.781
Δ $\%FEV_1/FVC$	-0.082	0.762	0.329	0.126
Δ blood serum TNFα	0.226	0.400	-0.106	0.630

FEV = Forced Evolutionary Virus; FVC = Forced Vital Capacity; TNFα = Tumor Necrosis Factor Alpha

Table 6. Correlation of LPS Endotoxin Level in Personal Dust with the Increase of Blood Serum TNFα Level and the Decrease of Lung Function, Year 2013

Variable	Cotton Spinning Workers		Village Administrators	
	r	LPS Endotoxin (p)	r	LPS Endotoxin (p)
Δ FVC	0.288	0.280	-0.418	0.047
Δ %FVC	0.221	0.411	-0.341	0.111
Δ FEV_1	0.736	0.001	-0.305	0.157
Δ $\%FEV_1$	0.712	0.002	-0.212	0.332
Δ $\%FEV_1/FVC$	0.324	0.221	0.110	0.618
Δ blood serum TNFα	0.551	0.027	0.085	0.701

FEV = Forced Evolutionary Virus; FVC = Forced Vital Capacity; TNFα = Tumor Necrosis Factor Alpha

All levels of personal dust generated in this research exceed the threshold limit value of 0,2 mg/m^3 considered safe for human to work up to eight hours per day or 40 hours per week, and this situation is strongly associated with a decrease in the workers' lung function. Continuous exposure of cotton dust may trigger lung reactions in the form of fibrosis, which causes breathing problems when the lungs undergo contraction.[7] Some studies also show a significant correlation between reduced lung function and high concentration of cotton dust.[8]

Research on the relationship between dust level and the prevalence of byssinosis and reduced lung function has been conducted by Jiang, et al.[9] in Guangzhou, China. With cotton dust level between 3.04 and 12.32, a decrease in the first second of a Forced Expiratory Wolume (FEV_1) was recorded at 21.8%, whereas FEV_1<80% was predicted at 6.1% and FEV_1/FVC<75% was at 4%.

Decreases in FEV_1 and $\%FEV_1$ levels are strongly associated with the presence of endotoxin in personal cotton dust. Exposure of endotoxin, which is a product of negative gram bacterium, has been proven to influence respiratory health. Epidemiological studies involving cotton farmers and textile workers have constantly revealed a strong relationship between responses to endotoxin exposure and acute lung function problems, which can be aggravated by the presence of chronic lung function disorders and non-allergic asthma.[10-12]

Airborne bacteria are one of the particulates which can enter human respiratory system. As part of their cell's outer membrane wall, LPS endotoxin will activate the production of proinflammatory cytokine by macrophage cells. Frequent exposure to organic dust containing LPS endotoxin will cause fever, bronchial hyperresposiveness, and the increase of proinflammatory mediators in blood serum, bronchoalveolar fluid, and nasal lavage. These reactions lead to the narrowing of human respiratory tract and affect lung function when measurement is being administered. This current research finds that the level of endotoxin in personal dust also has a significant correlation with the increase of blood serum TNFα level in cotton spinning workers. Relationship between LPS and the production of cytokine (such as TNFα) reveals LPS as the principal pathogen found in negative gram bacteria sepsis, whose symptoms include shock, coagulopathy, and multiple organ dysfunction.

Responses to systemic LPS exposure trigger the production of proimflammatory cytokine, such as tumor necrosisfactor (TNF)-, interleukin (IL)-1β, and interferon by host.[13] Injection of pure or lipid endotoxin to lab animals may trigger septic shock symptoms. Endotoxin in negative gram bacteria binds LPS-binding protein solution or the outer membrane of mononuclear cells. Interaction between monocytes, macrophages, and neutrophils leads the bacteria to discharge inflammatory mediators such as interleukin (IL), interferron (IF), platelet activating factor (PAF), and tumor necrosisfactor (TNF).[13]

Conclusions

This research concludes that there was a more significant decrease of lung function in cotton factory workers after one shift of work than in village administrators over the same period of time. No correlation has been found between the personal cotton dust level and blood serum TNFα level. However, if the level of personal cotton dust level increases, the workers' lung function will also decrease. If the level of LPS endotoxin in personal cotton dust increases, the level of blood serum TNFα will also increase. At the same time, the workers' lung function will also decrease at both $\Delta FEV_{1,}$ and $\Delta\%FEV_1$. Alarmed by the findings of this research, the management of the cotton factory should take both technical and administrative control measures. However, this paper argues that technical control is the best measure since it can eliminate both actual and potential dangers, so that harmful effects on workers can be mitigated. The main target of technical control measures is removing the actual dangers, and the effectiveness of the measures does not depend on workers' behaviors. An example of technical control measures is provision of a cotton dust absorber. In comparison, administrative control can be done by applying work-shift rotation and by providing a SOP for the workers. Besides those actions, the cotton factory management should also provide protective masks with a fiber density lower than the size of the hazardous cotton dust. Even more importantly, all of the workers involved should consider adopting a healthier lifestyle by reducing or avoiding the consumption of cigarette and doing regular exercise to increase their body immune system and improve their lung function.

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