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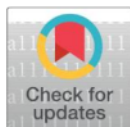
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The increasing of Interleukin 6 and Superoxide Dismutase 3 nasal wash in textile industry workers exposed by occupational air pollutant

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Abstract

Background: Occupational air pollution exposure in textiles industry which irritates the respiration system triggers the disturbance of the nasal mucosa. IL-6 is produced by monocytes and macrophages as a response for tissue damage. SOD-3 is extracellular enzymes protected the tissue from free radical and chronic inflammation. **Objectives:** To know if the occupational air pollution affects the increasing of IL-6 and SOD-3 level on nasal wash. **Methods:** this study used an analytical observational design with cross-sectional method. It was conducted on 40 male workers of garment industry in Bawen, Semarang, Indonesia. Divided into two groups: the dyeing and sizing area with 20 workers each group. The workers exposed to the occupational air pollutant for 5 years or more. The aquabidest will be injected to subject's nostril using the 20cc spuit. The nasal wash's sample on the 1.5ml eppendorf tubes will be examined using ELISA method to determine the IL-6 and SOD-3 level. The occupational air pollution indicators will be measured by EnviLab Semarang. **Findings :** The measurements of direct PM₁₀ and PM_{2.5} in dyeing and sizing area were higher than the regulatory limit. There was a significant difference in the IL-6 level in dyeing and sizing area ($p=0,010$). There was no significant difference in the SOD-3 level in dyeing and sizing area ($p=0,443$). There was a positive, moderate and significant correlation between IL-6 and SOD-3 in dyeing and sizing area ($r=0.320$). **Conclusion:** The study concluded that the occupational air pollution especially the PM₁₀ and PM_{2.5} affects the IL-6 and SOD-3 level in the textile industry workers' nasal wash. There is a significant correlation between the IL-6 and SOD-3 level in textile industry workers at dyeing and sizing area. There is a difference in the IL-6 level in dyeing and sizing area. But there is no significant in the SOD-3 level in dyeing and sizing area. The higher occupational air pollution inhaled will be compensated with the higher expression of IL-6 and SOD-3. The higher exposure of PM_{2.5} in the dyeing area results in the higher level of IL-6 and SOD-3.

Keywords: Interleukin 6; superoxide dismutase 3; occupational air pollution; ELISA

1 Background

Occupational air pollutant in textile industry irritates the respiration system which in turn causes the disturbance of the nasal mucosa⁽¹⁾. The occupational air pollution indicators are Carbon dioxide (CO), Nitrogen Oxide (NOx), Ozone (O₃), Lead (Pb), and particulate matter (PM)⁽²⁾.

Interleukin-6 (IL-6) is a glycoprotein produced by monocytes and macrophages due to the infection or tissue damage in immune system response. IL-6 will activate Toll-LIKE Receptors (TRLs) to produce IL-6 in the further chronic inflammation^(3,4).

Superoxide dismutase (SODs) is an antioxidant enzymes produced by human immune system for oxidation homeostasis. SOD-1 and SOD-2 are the intracellular enzymes work in cytoplasm and mitochondria. SOD-3, as known as Extracellular Superoxide Dismutase (ECSOD), is an extracellular enzyme found in lung and tissues. The distribution of SOD-3 in the extracellular compartment indicates that the enzyme is protecting against free radical damage and chronic inflammation. In the state of stress oxidative, the production of free radical damages the extracellular matrix^(5,6).

Enzyme-Linked Immunosorbent Assay (ELISA) Method is used to measure the IL-6 and SOD-3 level. ELISA is a plate-based testing technique designed to detect and measure peptides, proteins, antibodies and hormones. ELISA test can be done in a relatively fast time with high sensitivity and specificity^(7,8).

The objective of this study is to know if the occupational air pollution exposure affects the increasing of IL-6 and SOD-3 level on nasal wash in textile industry workers.

2 Material and method

2.1 Samples and Variable

This study used analytical observational design with cross-sectional method. The subjects of this study were 40 male textile industry workers in PT. APAC INTI Corpora, Bawen, Semarang. Divided into two groups: the dyeing and sizing area with 20 workers each group. Subjects were selected according to inclusion and exclusion criteria using a questionnaire.

1. Inclusion criterias:

- (a) PT. APAC INTI Corpora's dyeing and sizing male workers.
- (b) In a good condition.
- (c) 5 years or more of service.
- (d) Signing the informed level.

2. Exclusion criterias: history of lung and heart disease

The aquabidest will be injected to subject's nostril using the 20cc sput. The nasal wash's sample in the eppendorf tubes will be examined using ELISA method to determine the IL-6 and SOD-3 level. The ELISA fine test will be measured by Biomolecular laboratory in Semarang. The occupational air pollution indicators used to determine the air quality of the garment industry in Bawen, Semarang, Indonesia will be measured by Environmental Laboratory and Consulting Service Company (Envilab Semarang). This study approved by health research committee of Medical Faculty Diponegoro University with ethical exemption number No.194/EC/KEPK/FK UNDIP/V/2019.

2.2 Data Analysis

The data normality test from each group was analyzed using the Saphiro-wilk test. The Mann Whitney test was used for the abnormally distributed data. The difference test is significant if $p < 0,05$. The data's correlation test was used the Spearman's correlation test. The correlation is significant if $p < 0,05$.

3 Result

3.1 Respondent characteristics

The characteristics of respondents in this study can be seen in Table 1 .

Table 1. Respondent Characteristics

Characteristics	n	%	Mean ± SD (min-max)
Gender	40	100%	-
Male			
Female	0	0%	
Age	40		39.93 ± 8.166 (19-50)
Year of service	40		17.34 ± 8.078 (5-27)
Duration of work 8 hours	40	100%	-
Length of work			
5 days	6	15%	5.88 ± 0.404 (5-7)
6 days	33	82,5%	
7 days	1	2,5%	
Other occupation exposed by irritants			
Yes	2	5%	-
No	38	95%	
Respiratory disorder			
Yes			-
No	40	100%	
Smoking history			
Yes	25	62,5%	-
No	15	37,5%	

3.2 The indoor occupational air quality in dyeing and sizing area

Table 2. The indoor air quality measurement in dyeing and sizing area.

No.	Parameter	Area	Test Result	Regulatory limit	Unit	Method
1.	Sulphur dioxide (SO ₂)	Dyeing	<0.0003	0.5	ppm	IKM-EI-SML-21
		Sizing	<0.0001			
2.	Nitrogen Dioxide (NO ₂)	Dyeing	<0.0032	0.2	ppm	IKM-EI-SML-22
		Sizing	<0.0032			
3.	Oxidant (O ₃)	Dyeing	<0.007	0.08	ppm	IKM-EI-SML-24
		Sizing	<0.007			
4.	Hydrogen Sulfide (H ₂ S)	Dyeing	<0.012	1	ppm	IKM-EI-SML-6
		Sizing	<0.012			
5.	Ammonia (NH ₃)	Dyeing	0.0001	25	ppm	IKM-EI-SML-23
		Sizing	0.0005			
6.	Carbon Monoxide (CO)	Dyeing	<1	25	ppm	NIOSH 6604:1996
		Sizing	<1			
7.	Lead (Pb)	Dyeing	<0.0003	0.05	mg/m ³	OSHA ID-121:2002
		Sizing	<0.0003			
8.	Dust	Dyeing	5.36	10	mg/m ³	SNI 16-7058-2004
		Sizing	1.33			
9.	PM ₁₀	Dyeing	64	50	μg/Nm ³	IKM-EI-SML-28 (Particle counter)
		Sizing	72			
10.	PM _{2.5}	Dyeing	34	25	μg/Nm ³	IKM-EI-SML-28 (Particle counter)
		Sizing	33			
11.	Noise	Dyeing	65.4	dBA	SNI	7231:2009
		Sizing	66.3			

The results showed that in the dyeing and sizing area, the direct PM10 and PM2.5 measurements is above the regulatory limit standard for 24-hour. The SO₂, NO₂, O₃, H₂S, NH₃, Dust, CO, Lead, and noise are below the regulatory limit as shown in Table 2 .

3.3 The difference test of IL-6 and SOD-3 level in dyeing and sizing area

From the Table 3 , it can be seen that the difference test using Mann Whitney test is showed a significant difference of IL-6 level between dyeing and sizing area with p=0.010 (p < 0.05). The difference test of SOD-3 between the dyeing and sizing area did not show a significant result with p=0.433 (p < 0.05).

Table 3. The difference of IL-6 and SOD-3 level in dyeing and sizing area

Variable	Area		p [‡]
	Dyeing (Mean±SD)	Sizing (Mean±SD)	
IL-6	12.786±1.983	12.591±8.137	0.010
SOD-3	4.490 ± 2.771	3.644±1.694	0.433

3.4 The correlation test between IL-6 and SOD-3 level in dyeing and sizing area

The correlation between IL-6 and SOD-3 level in dyeing and sizing area can be seen in Table 4 . From the Table 4 it can be seen that the correlation test using spearman’s test between IL-6 and SOD-3 level in dyeing and sizing area showed a significant correlation with p=0.044 (p < 0.05). The correlation coefficient showed a positive moderate strength between the IL-6 and SOD-3 level in dyeing and sizing area with r=0.320.

Table 4. The correlation between IL-6 and SOD-3 level in dyeing and sizing area

Variable	SOD-3		Descriptionp
	†r		
IL-6	0.044	0.320	Significant, positive, moderate

* Significant (p< 0.05);[‡] Spearman’s

4 Discussion

4.1 Air pollutant toxicity in dyeing and sizing area

According to WHO, the 24-hours regulatory limit for PM10 is 50 µg/Nm³ and 25 µg/Nm³ for PM2.5. The results of the SO₂, dust, and PM2.5 measurement in the dyeing area were higher than the sizing area. The results of the NO₂, H₂S, CO, and lead measurement in dyeing and sizing area were the same. The results of the NH₃, noise and PM10 measurement in dyeing area were lower than the sizing area. The high level of PM10 and PM2.5 in the dyeing and sizing area is not safe for the workers.

PM has more serious impact on human health compared to the larger particles. PM is categorized according to mean aerodynamic diameter with sizes ranging from coarse particles (2.5–10 µm in diameter) to the smaller fine (<2.5 µm) and ultrafine (<0.1 µm) particles. PM can be inhaled to the lower respiratory airway. The smaller the size, the deeper the PM can be inhaled to the lower respiratory airway. The inhaled PM will be deposited in the lungs and damage the alveoli⁽⁹⁻¹¹⁾.

Occupational air pollutant may result in adverse health effect in the exposed textile industries workers, even if it is in a small amount and does not exceed the occupational exposure limits. It may deposited in the workers’ respiratory tract when it is inhaled. The exposure of respiratory tract to the occupational air pollutant can cause hyperactivity of mucouse membrane resulting in the inflammation of the mucosa, obstruction, restriction, and manifest the decreasing of the pulmonary function. The textile industries workers’ smoking habit also may impact on their health.^(12,13)

4.2 The difference of IL-6 and SOD-3 level in dyeing and sizing area

The analysis of the data showed a significant difference of IL-6 level between dyeing and sizing area with p=0.010 (p < 0.05). The difference test of SOD-3 between the dyeing and sizing area did not show a significant result with p=0.433 (p < 0.05). The results of the IL-6 and SOD-3 measurement in the dyeing area were higher than the sizing area so it was proved the hypothesis of this study that there is a difference between IL-6 and SOD-3 level.

The history of the smoking habit in the subjects was one of the confounding factors in this study. The smokers tend to produce IL-6 level in the respiratory tract epithelium compared to the non-smokers. The interaction of smoking with IL-6 can affect the disease of the respiratory system. From a study by⁽¹⁴⁻¹⁶⁾ a moderate positive correlation was found between IL-6 and smoking habits. The higher the intensity of the smoking, the higher IL-6 level will be produced.

Cigarette smoke exposed increases oxidative stress which can trigger the inflammation in the lung. The inflammation will spread systemically to the blood vessels and cause a decreasing of the body's endogenous antioxidant capacity. The body produces SOD-3 as an antioxidant which can protect the body from the oxidative stress coming from the cigarette smoke. Poor awareness in smoking habit contributed into smoke-induced lung disease, including COPD, lung cancers and respiratory infections.⁽¹⁷⁾

From the study conducted by^(18,19) found that there is a decreasing expression of antioxidant enzymes such as SOD-2, SOD-3, and PRDX4 in mice whose lungs were exposed to PM2.5. This study proving why the SOD-3 level are lower than the IL-6 level.

4.3 The correlation between IL-6 and SOD-3 level in dyeing and sizing area

The results of the data analysis with $p=0.044$ showed a significant correlation between IL-6 and SOD-3 measurements in the dyeing and sizing area. The correlation coefficient showed a positive moderate strength between the IL-6 and SOD-3 level in dyeing and sizing area with $r=0.320$. The positive correlation from the data analysis means if there is an increasing amount of IL-6 level, the SOD-3 level will also increased.

The IL-6 level indicated a sign of an ongoing inflammatory process in the tissues⁽²⁰⁾. The expression of SOD-3 in the extracellular compartment indicates that the enzymes in protecting the body against free radical damage and chronic inflammation. The inflammation process will disturbed the SOD-3 activity⁽²¹⁾. The low SOD-3 level showed that the antioxidant activity in the body can not protect against free radical damage and inflammation.⁽²⁾⁽²⁾

From a study by⁽²²⁾ showed that the expression of IL-6 caused by the air pollutant will be suppressing the TNF- α , SOD-3 and ROS. From the study, the *Nephrolepis exaltata*'s herbal mask is protect the respiratory airways of the textiles industry workers from the air pollutant.

4.4 The correlation between IL-6 with PM10 and PM2.5 in dyeing and sizing area

From the study, there was a high chronic PM10 and PM2.5 exposure in the dyeing and sizing area that exceeds the regulatory limit. The body's immune system will respond to this chronic exposure with the expression of pro-inflammatory cytokines such as IL-6.

The previous study by⁽²³⁾ proved that particulate matter (PM) contains polyaromatic hydrocarbons which triggers the inflammatory. It proved that PM2.5 stimulus in mice increased the IL-6 expression by binding to TLR5 at 3 hours and 24 hours of measurement. These results are compatible with the result of the study that there is a correlation between the level of IL-6 with the PM2.5 exposure in the dyeing and sizing area.

The same result obtained from⁽²⁴⁾ showed that there is an increasing amount in Thioredoxin (Trx) translocation to the nucleus induced by PM2.5 toxicity. Inhalation of PM2.5 to the lungs triggers oxidative stress and inflammatory reactions which cause a lot of lung diseases.

From the study of⁽²⁵⁾ there is a prevalence of cough and mucus. There is a significant correlation between the IL-6 and PM10 at the bus driver in Klang Valley. These results are compatible with the result of the study that PM10 exposure in the dyeing and sizing area triggers the expression of IL-6.

4.5 The correlation between SOD-3 with PM10 and PM2.5 in dyeing and sizing area

The result of the study found that there is a correlation between SOD-3 level with PM10 and PM2.5 exposure in dyeing and sizing area. These results are compatible with the result from the previous study conducted by⁽²⁶⁾ PM10 induced inactivation of antioxidant enzymes in SOD, catalase, GR, and GST. PM10 triggers the expression of ROS which affect the SOD degradation. In⁽²⁷⁾ the study by showed that PM2.5 induces the expression of inflammatory cytokines such as GM-CSF, TNF- α , IL-13, eotaxin, IL-6 and IL-8. These results indicate that PM2.5 induces oxidative stress and inflammatory responses in RPMI 2650 cells and causes nasal epithelial barrier dysfunction.

According to⁽²⁸⁾ the study conducted by PM10 can cause oxidative stress in mice. From the study, there was a decrease in SOD activity in serum, lung tissues, and heart tissues in wistar rats induced by PM10 by 7.5 mg/kg and 15 mg/kg. These results proved that the exposure of PM10 and PM2.5 make the expression of SOD-3 yields lower than IL-6 because there is an imbalance of antioxidant and oxidant ability in the circulatory system.

5 Conclusion

The occupational air pollutant especially the PM10 and PM2.5 affects the IL-6 and SOD-3 levels in the textile industry workers' nasal wash. There is a significant correlation between the IL-6 and SOD-3 level in textile industry workers at dyeing and sizing area. There is a difference in the IL-6 level in dyeing and sizing area. But there is no significant in the SOD-3 level in dyeing and sizing area. The higher occupational air pollution inhaled will be compensated with the higher expression of IL-6 and SOD-3. The higher exposure of PM2.5 in the dyeing area results in the higher level of IL-6 and SOD-3. Occupational air pollutant may result in adverse health effect in the exposed textile industries workers, even if it is in a small amount and does not exceed the occupational exposure limits.

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