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Research Article

Pulmonary Function and Malondialdehyde (MDA) Content in Blood Due to Chromium Exposure Among Tannery Workers in Sukaregang, Garut

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Abstract

Background and Objective: Chromium (Cr) has been widely used in many industrial application. Chromium application in tannery industry has potential adverse effects to workers. This study was aimed to determine the effect of chromium exposure to health effects by measuring malondialdehyde (MDA) content in blood and pulmonary dysfunction among tannery workers. **Methodology:** A total of 25 workers were included. Ambient air chromium was measured in 6 different locations. Blood and urine were collected to measure MDA content and total chromium. The MDA was determined by using spectrophotometric assay TBAR reaction method and chromium was determined by using graphic furnace Atomic Absorption Spectrometry (AAS). Spirometry was used to evaluate pulmonary dysfunction. **Results:** Result revealed that urinary Cr in workers was $19.65 \mu\text{g L}^{-1}$ ($n = 25$). Twenty five percent of workers urinary Cr was above Biological Exposure Indices (BEI) ACGIH ($\geq 25 \mu\text{g L}^{-1}$). Malondialdehyde (MDA) was not significantly different among high exposed workers ($\text{Cr} > \text{BEI}$) and low exposed workers ($\text{Cr} < \text{BEI}$) ($p = 0.716$), ($0.118 \text{ nmol mL}^{-1}$) and ($0.124 \text{ nmol mL}^{-1}$), respectively. **Conclusion:** It had been concluded that workers had high urinary chromium. It showed high chromium exposure in occupational ambient air in tannery industry. Chronic exposure of chromium could increase oxidative stress status and adverse health effects.

Key words: Malondialdehyde, chromium, tannery, pulmonary dysfunction

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chromium (Cr) is a heavy metal most widely used in industrial processes. It is widely used mainly in metal plating, paint, stainless steel manufacture, development of pigments, tannery, catalysts, materials surface smoothing and refractory (Tziritis *et al.*, 2012; Soudani *et al.*, 2011; Pellerin and Booker, 2000). Leather tannery is one of the industries using large amounts of chromium. It is the process of converting rawhide into leather basic material by using chromium in the form of Cr(III) hydroxide sulphate ($\text{Cr}(\text{OH})\text{SO}_4$) and the addition of other chemical compounds such as formaldehyde, glutaraldehyde and heavy lubricants (Hylli, 2013). Tanning is the most important process because this process allows the leather to be durable, wet resistant and prevents it from decay (Bayramoglu *et al.*, 2012). Workers in the tanning industry can be exposed to chromium through air, in contact with skin or ingested through food in the workplace. Chromium exposure can be risky to the workers' health (Khan *et al.*, 2012).

Chromium exposure can be a serious problem since it is known to have characteristics that are highly toxic to living organisms (Wise and Wise Jr., 2012). The WHO and US Environmental Protection Agency (EPA) have determined that hexavalent chromium ($\text{Cr}(\text{VI})$) is a chemical compound that is carcinogenic to humans particularly can cause respiratory tract cancers. The $\text{Cr}(\text{VI})$ can cause irritation to the respiratory tract after inhalation exposure and may increase the risk of lung cancer (Hylli, 2013). In addition, chromium is known to cause perforation in the nasal septum, irritation and inflammation of the skin. In some cases with high enough exposure, it is reported damage to kidney, liver, lung edema, erosion of the teeth and epigastritis (ATSDR., 2012).

Repeated chromium exposure increases the risk of pulmonary tissue toxicity. Research conducted on 24 workers of chrome plating, it was known increased levels of chromium in workers' exhaled breath condensate, EBC from $5.3 \mu\text{g L}^{-1}$ to 6.4 mg L^{-1} . Increasing concentrations of inhaled chromium would also indicate increasing health risks in the respiratory organs (Caglier *et al.*, 2006). Research conducted in Kanpur, Pakistan, 197 tannery workers showed that about 16.7% workers had respiratory system disorders. The occurrence of respiratory system disorder was caused by high exposure to chromium in the environment and behavior that did not work according to standards (Rastogi *et al.*, 2008).

Effect of chromium on the disease incidence occurs through a mechanism of oxidative stress in the body (Bagchi *et al.*, 2001). Chromium entering into the body will undergo reduction and oxidation reactions as a result of metabolic processes. These reduction and oxidation can form

free radicals in the form of Reactive Oxygen Species (ROS) or Reactive Nitrogen Species (RNS) play role in the incidence of the disease. The occurrence of oxidative stress can be characterized by malondialdehyde (MDA) content. The MDA is a compound resulted from the process of lipid peroxidation that occurs when there is reaction between free radical and unsaturated fatty acid composing phospholipid. The MDA formation can be transmitted to tissues throughout the body. The MDA on the network can form covalent bonds able to modify the molecule structure, so that it can alter the character of the molecule. The MDA has the properties of high cytotoxicity, mutagenic, inhibitors at various enzyme, inhibitors of DNA replication and playing a role in the incidence of respiratory disease (Klaunig *et al.*, 2011; Valavanidis *et al.*, 2006).

MATERIALS AND METHODS

This study was conducted using cross-sectional design of the study with the population of tannery workers. This study was conducted at the Tannery Industry Center of Sukaregang, under the responsibility of the Sub-Unit of Tannery of Industry Department of Garut district, West Java, after passing through ethic review phase from the Ethics Committee of FKM UI (Faculty of Public Health, Universitas Indonesia).

Samples: The samples were workers selected through pre-defined criteria. The samples were selected by determining the inclusion and exclusion criteria. The samples were 25 people working at the tannery actively registered in the Sub-Unit of Tannery of Garut district and 25 non-workers.

Individual characteristic data: Individual characteristic data included personal data, i.e., name, gender, age, length of work, duration of work and health conditions. In addition, it was also collected the data on individual habits including smoking, alcohol drinking habit, exercise habit and use of personal protective equipment during working. The data was collected with interview using questionnaire instrument.

Air chromium: Air chromium samples were taken in some industry locations of tannery. Chromium sampling point in air was taken at six different points. The collection of chromium concentration in the air referred to NIOSH (1994) Manual of Analytical Methods (NMAM), 4th edition. No. 7024 in 1994 to assess the total chromium in the air. For the calculation of chromium concentration, the samples were analyzed using Atomic Absorption Spectrophotometry (AAS) referring to Perkin-Elmer method.

Table 1: Worker characteristics (n = 25)

Variable	Workers	
	No.	%
Sex		
Male	25	100
Female	0	0
Age (years old)		
<25 years old	5	20
25-50 years old	20	80
>50 years old	0	0
Length of work (years)		
≥10 years	9	36
<10 years	16	64
Smoking		
Yes	21	83
No	4	17
Wearing mask		
Yes	3	12
No	22	88

Table 2: Measurements result of Cr concentration in the workspace air

Area/rooms	Cr concentration ($\mu\text{g m}^{-3}$)	
	Results	TLV
Location A (KLM)	500	11.79
Location B (KLM)	500	7.86
Location C (BPIP)	500	5.17
Location D (BPIP)	500	3.94
Location E (PS)	500	5.19
Location F (LJ)	500	7.84

Table 3: The total chromium levels of urine ($\mu\text{g L}^{-1}$) and malondialdehyde (MDA) (nmol mL^{-1}) of blood in the worker group (n = 25)

Tannery workers					
Sample	Mean	Min.	Max.	SD	p-value
Urinary Cr*	19.650	0.200	91.000	2.440	0.486
MDA	0.122	0.087	0.245	0.036	

*Undetectable value was interpreted as $\frac{1}{2}$ LOD = $0.2 \mu\text{g L}^{-1}$ SD: Standard Deviation

Urinary chromium: Urine samples were collected from the entire sample of 25 urine of workers and 25 urine in the control. The urine was taken after respondents agreed the informed consent read and explained by the researcher. The urine of workers was taken at the end of the working shift, that was from 03.00 PM until 5:00 PM. The urine in the control group was taken at the same range of time with the worker group, namely from 03.00 PM until 5:00 PM. Chromium levels in urine were measured by the method described by Kuo *et al.* (2004). Levels of chromium in the sample were then measured using Atomic Absorption Spectrophotometry (AAS), ICP Spectrometer ICAP 7000 series brand with the technique of graphite furnace (Perkin-Elmer Model) and Zeeman correction.

Malondialdehyde (MDA) in the blood: MDA measurement was carried out using the method described by Wills (1987). Measurement of MDA contents was carried out by analyzing

blood serum taken from worker samples. The blood taken was median cubital vein blood by approximately 10 mL per sample. The workers chosen as samples were given informed consent to obtain approval engaged in the research and taken some material from their body.

Pulmonary function: Examination of pulmonary function using spirometry tool referred to the method used (Miller *et al.*, 2005). Then to determine the pulmonary function condition of tannery workers, it referred to the regulation of the Ministry of Manpower and Transmigration No. PER.25/MEN/XII/2008 on Guidelines for Diagnosis and Disability Assessment for Occupational Injuries and Diseases.

RESULTS

The selected sample of workers in accordance with the criteria involved 25 male workers who had the productive age ranging from 20-50 years old (80%). About 88% workers did not wear masks during working (Table 1).

The total concentration of chromium (Cr) in the air of workspace of several measurement locations varied. Although, the measurement results varied, the results were still far from the Threshold Limit Value (TLV) established by the Minister of Manpower and Transmigration Republic of Indonesia as detailed in the Regulation number PER-13/MEN/X/2011 on the threshold limit value of physical and chemical factors in workplace. In the regulation PER-13/MEN/X/2011, it was stated that the total Cr maximum concentration allowed in the workplace was of $500 \mu\text{g m}^{-3}$ or 0.5 mg m^{-3} (Table 2).

The Threshold Limit Value (TLV) was based on PER-13/MEN/X/2011 on the threshold limit value of physical factor and chemical factor in workplace.

The examination of chromium level in the worker urine (n = 25) was detected by the value range of $4.2\text{-}91 \mu\text{g L}^{-1}$ with not detected value (TT) in accordance with the minimum limit of examination method as many as 4 samples (Table 3).

The average level of total chromium in the urine of worker group detected was $19.65 \mu\text{g L}^{-1}$. Based on these results, there were 25% samples of worker who had urinary chromium levels above the value of Biological Exposure Index (BEI) (urinary Cr > $25 \mu\text{g L}^{-1}$) (Table 3). The MDA is an indicator that can be used to indicate the activity of oxidative stress in the body. The MDA content in whole blood samples were detected in the range of $0.087\text{-}0.245 \text{ nmol mL}^{-1}$ with the mean of $0.122 \text{ nmol mL}^{-1}$. The correlation between urinary chromium levels and MDA contents in the blood showed no statistically significant relationship between urinary chromium and MDA (p = 0.486) (Table 3).

Table 4: Pulmonary dysfunction and chromium concentration in occupational ambient air ($\mu\text{g m}^{-3}$)

Pulmonary function	Air Cr ($\mu\text{g m}^{-3}$)	p-value
Pulmonary dysfunction	8.27	0.161
Normal	6.17	

Table 4 shows that the workers indicated to have pulmonary dysfunction exposed by the chromium are higher than the normal worker, i.e., respectively by 8.27 and $6.17 \mu\text{g m}^{-3}$. However, the statistical test results show no significant differences between Cr exposure in the air in workers experiencing pulmonary dysfunction and workers who do not have pulmonary dysfunction ($p = 0.161$).

DISCUSSION

Chromium is a heavy metal widely used in industrial processes, especially in tannery industry. The chromium used can be released into the environment through waste and air in a variety of processes such as scrapping and heating. The results of this study find that the concentration of chromium in the air of workspace is still below the threshold limit value determined based on the total chromium standard of OSHA by $500 \mu\text{g m}^{-3}$ for 10 working hours. The results of the review carried out by Gibb *et al.* (2000) states that the value of permissible exposure limit (PEL OSHAL) of CrO_3 was changed from $100\text{--}50 \mu\text{g m}^{-3}$. Moreover, NIOSH REL (recommended exposure limit) recommends much lower, that is equal to $1 \mu\text{g m}^{-3}$ (Costa and Klein, 2006).

This figure is quite small compared to that detected by Balachandar *et al.* (2010) that measure the chromium exposure in tannery in South India by 101 and $89 \mu\text{g m}^{-3}$. Chromium used in the tannery process is Cr(III) with low toxicity, but Cr(III) under certain conditions in the body can be oxidized into Cr(VI) that is more toxic. The formation of Cr(VI) in the body can initiate the formation of free radicals and increase the activity of oxidative stress (ATSDR., 2012).

The results of chromium measurement in this study show the high levels of chromium in workers urine. The average level of urinary chromium in worker samples is detected by $19.65 \mu\text{g L}^{-1}$ ($n = 25$). The chromium level value that is not detected is set at half of LOD value, i.e., $0.2 \mu\text{g L}^{-1}$. 25% workers have urinary chromium levels that exceed the value of Biological Exposure Index (BEI), that is $25 \mu\text{g L}^{-1}$. Research conducted by Khan *et al.* (2012) to tannery workers in Pakistan shows the median of urinary Cr by $50.6 \mu\text{g L}^{-1}$ in workers is higher than the control by 5 mg L^{-1} . A study conducted by Balachandar *et al.* (2010) showed that urinary chromium levels in tannery workers differ among workers

exposed directly, indirectly and controls, respectively for 2.11, 1.81 and $0.54 \mu\text{g L}^{-1}$. Of the urinary chromium levels in electroplating workers exposed to chromium, it is obtained the mean of $12.81 \mu\text{g L}^{-1}$ ($0.20\text{--}144 \mu\text{g L}^{-1}$) higher than the control by $0.99 \mu\text{g L}^{-1}$ ($0.2\text{--}3 \mu\text{g L}^{-1}$) (Kuo *et al.*, 2004).

Urine is one of the most important excretion media in removing heavy metals from the body. When chromium enters the body, chromium will be distributed immediately throughout the body via the blood with the target of organs such as the lung, liver and kidney (Feng *et al.*, 2015). Several studies show significant relationship between levels of chromium in the urine and high levels of chromium in the blood. In addition, chromium levels of urine and blood is correlated with the chromium concentration of occupational ambient air (Zhang *et al.*, 2008; Khan *et al.*, 2012).

The occurrence of oxidative stress can be characterized by malondialdehyde (MDA) content analysis. The MDA is a compound resulting from the process of lipid peroxidation that occurs at the time of reactions between free radical and unsaturated fatty acid composing phospholipid (Klaunig *et al.*, 2011). The MDA contents in the blood are detected ranging from about $0.087\text{--}0.245 \text{ nmol mL}^{-1}$ with an average of $0.122 \text{ nmol mL}^{-1}$. There is no significant correlation between the urinary chromium levels and MDA contents in the blood ($p > 0.05$).

This MDA content is quite also small compared to other studies. In the similar study by Balachandar *et al.* (2010), it is showed a significant difference between the workers and the control group. The MDA content shows significant results in tannery and welding workers exposed to chromium compared to the unexposed group respectively $1.42 \mu\text{mol g}^{-1}$ creatinine, $1.67 \mu\text{mol g}^{-1}$ creatinine and 0.81 mol g^{-1} creatinine (Goulart *et al.*, 2005). Research conducted Ambreen *et al.* (2012) in the tannery workers in India shows that the MDA contents of workers have a significant relationship with urinary chromium levels ($p < 0.0001$).

However, in some other studies MDA also does not show significant relationship with exposure. Research conducted by Erden *et al.* (2014) regarding BpA exposure and MDA contents does not show any significant difference between BpA-exposed workers and the control, respectively 1.02 and $1.06 \text{ nmol mL}^{-1}$. Similar results were shown by study conducted by Kasperska-Zajac *et al.* (2008) examining the MDA contents in COPD patients and the control, detected, respectively, 1.6 and 2.4 nmol mL^{-1} .

No significant relationship between urine and MDA contents in this study may be due to the presence of individual risk factor not measured. The MDA contents can be

influenced by risk factors such as nutrition, lifestyle habits and individual susceptibility (Caglier *et al.*, 2006). Micronutrients such as vitamin C and E may increase the levels of antioxidants in the network and protect the cell membrane against oxidative stress. People who are deficient in vitamin C and E are correlated with increased levels of oxidative stress in the body (Valko *et al.*, 2006). Related research is conducted by Shohag *et al.* (2012) examining the relationship between the levels of oxidative stress and status of vitamin C and E in patients with obsessive-compulsive disorder. The results show that the low levels of vitamin C and E are correlated significantly with the increase in MDA levels in the blood.

Chromium levels are higher than workers who do not experience decline in pulmonary function (normal). Workers indicated by decreased lung capacity are based on the median value of the data, exposed by chromium at $6.33 \mu\text{g m}^{-3}$. Meanwhile, the workers indicated to have pulmonary dysfunction based on symptoms suffered due to chromium exposure is $7.71 \mu\text{g m}^{-3}$. However, there is no statistically significant difference between the chromium concentration and the indication of pulmonary dysfunction in tannery workers ($p = 0.161$).

Decline in pulmonary function is due to the air flow resistance in respiratory tract that is not fully reversible (back to normal). Decline in pulmonary function is a form of condition of abnormality in the respiratory system. Pulmonary dysfunction is a condition in which there is abnormality in the respiratory system ventilation. The ventilation process on the respiratory system is the process of entry of air into the lungs as well as the release of carbon dioxide gas from alveoli. This ventilation disturbance causes decrease in pulmonary function, especially in the case of lung capacity. This condition is often caused by viruses, bacteria, dust and other particles (Mader, 2001; Plog and Quinlan, 2002).

Decline in pulmonary function is affected by many factors, such as age, smoking habit, space air pollution, exposed to dust or chemicals and ambient air pollution. A similar study was conducted in India on 130 people working in tannery and 130 people working in the office. Spirometry test results showed that forced expiratory flow rate (FEF 25-75%) in the tannery workers decreased as many as 56 people (43%). A total of 22 people (17%) of the 130 workers in the tannery industry experienced restrictive pulmonary dysfunction and 14 people (11%) experienced mixed pulmonary dysfunction, restriction and obstruction (Chandrasekaran *et al.*, 2014). The similar results were also found in other studies, finding that 16.7% of tannery workers exposed by chromium experienced respiratory problems (Rastogi *et al.*, 2008).

CONCLUSION

This study shows the chromium exposure potential in tannery occupational environment based on the levels of chromium detected. The levels of chromium are detected in all sampling points, but still below the safe limit. Urinary chromium levels in workers are detected to have high levels and it is known as many as 25% of workers exceed the value of Biological Exposure Indices (BEI), chromium urine is higher than $25 \mu\text{g L}^{-1}$. There is no statistically significant correlation between urinary chromium levels and MDA in the blood. This may be caused by the presence of other influencing risk factors such as nutrients, lifestyle and individuals vulnerability who are not examined in this study. There is incidence of pulmonary dysfunction on some workers, but it does not show any significant correlation with the levels of chromium in the occupational ambient air. Workers are expected to implement a working system in accordance with the rules of Occupational Health and Safety (OHS) and the use of personal protective equipment, especially masks to minimize the risk due to chromium exposure in the workplace.

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