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### Effect of Mercury Exposure on Renal Function and Hematological Parameters among Artisanal and Small-scale Gold Miners at Sekotong, West Lombok, Indonesia

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### Introduction

Artisanal and small-scale gold mining (ASGM) activities in West

Nusa Tenggara (WNT) province began in mid-2009. Hydrargyrum (mercury) amalgamation has been the most common method used to recover gold. Besides the availability of mercury (Hg), this technique is widely used because it is considered to be efficient, effective and affordable.1 In Indonesia, at least 250,000 miners were estimated to be directly involved in more than 1,000 areas of ASGM, spread throughout the country.<sup>2</sup> This report quantified 713 locations of illegal small-scale mining throughout Sumatra, Java, Kalimantan and Sulawesi.<sup>2</sup> In addition, there are around 300,000 to 500,000 people affected by the ASGM activities, the majority of which are non-registered, informal operations

*Background.* Mercury is a toxic metal with effects on human health ranging from acute to chronic in a very short time of exposure. Artisanal and small-scale gold mining (ASGM) is the main 617 ce of direct human exposure to mercury. Human exposure to mercury (Hg) can occur through both direct inhalation of mercury vapor and consumption of material taken from contaminated areas. To protect the health of ASGM workers and surrounding communities, a health assessment of mercury exposure and its effects is urgently needed. However, analysis of hair and urine samples as a proof test for mercury toxicity is very expensive. Therefore other tests must be considered to identify the first symptoms of mercury toxicity in refers and the surrounding community.

*Objectives.* The present study aimed to determine the effects of mercury exposure on renal function along with the hematological parameters of gold miners and the community as a first indication of mercury exposure symptoms.

Methods. The study was designed as a purposive field sampling study and was conducted in 3 main villages in Sekotong District, West Lombok Regency, West Nusa Tenggara Province, Indonesia. The 100 subjects were miners that have been exposed to mercury for at least 5 years and their wives and children (non-miners) who lived around the gold processing area. Blood and urine samples were then obtained from the subjects. The miners and nonminers were questioned about their mercury exposure over the previous 5 years, duration of exposure, and how mercury was handled in their daily life. Blood and urine samples were collected at the time of the study, around 10 ml of urine and 0.1 ml of blood (2 drops) were collected per subject. In order to determine the parallel results between the blood-urine and hair result, hair from the miners was collected at a different time for analysis.

*Results.* The results showed that the subjects had low proteinuria, hemoglobin and hematocrit concentrations as a consequence of chronic mercury intoxication. This finding was parallel with results of high mercury concentrations in urine  $(>7 - 273.3 \ \mu g/l)$  and miners' hair  $(>1 - 12.93 \ \mu g/g)$ . Miners and non-miners in the exposure area were found to have proteinuria levels of more than 0.3 g/L. Proteinuria  $(\geq 0.3 \ g/L)$  was observed in 92.6% of miners and 72.4% of non giners.

*Conclusions.* The results of the present study suggest that urinalysis of proteinuria and hemoglobin values can be used as a screening test to detect renal impairment due to mercury no xication.

Competing Interests. The authors declare no competing financial interests. Keywords. ASGM, proteinuria, hemoglobin, hematocrit J Health Pollution 9: 25-32 (2015)

located in remote areas in Indonesia.3

On Lombok Island alone, in 2012 there were around 22,500 people estimated to be involved in this activity.<sup>4</sup> The amalgamation method of ASGM results in Hg emissions that enter the environment in several ways. Mercury is unintentionally or intentionally spilleopnto the ground or agriculture land. Atmospheric transport and

deposition at normal temperature is another common way for Hg to enter many water systems. In addition, is often discharged together with other wastes into inadequate tailings ponds, or directly disposed into rivers and water systems. Moreover, when rifying the amalgam by burning, vaporized Hg is released into the atmosphere.<sup>5</sup> In addition, mercury gets into the human body through the food chain when fish are eaten or used in agricultural products.<sup>6-8</sup> Many studies have shown that mercury emissions into the environment affect human health.9-13

### Mercury is a highly toxic metal

that directly affects the nervous and cardiovascular system. Nausea, vomiting, diarrhea and severe kidney damage may occur due to exposure to high doses of mercury over a short period of time. Hallucinations, memory loss, nerve damage and the inability to concentrate can also occur. Furthermore, mercury toxicity symptoms include tremors, loss of dermal sensitivity, and slurred speech.<sup>14,15</sup>

Mercury exposure can also come through inhalation of inorganic metallic mercury, ingestion of inorganic complexed mercury, or ingestion of organic forms of mercury. The factors regarding the occurrence and severity effects of mercury on human health include: the chemical form and dose of mercury; the age or the developmental stage of the person exposed, and the duration and exposure route, including inhalation, ingestion, and dermal contact. Fish consumption patterns can also increase the chance of mercury exposure when fish and seafood are contaminated with mercury. Mercury toxicity interferes with vital body systems: the nervous system, kidney function, and cardiovascular system. It has been

AbbreviationsASGMArtisanal and small-scale<br/>gold miningHgMercury<br/>Mercury<br/>SDHbHemoglobinSDStandard deviationHbHemoglobinWHOWorld Health OrganizationHCTHematocritWNTWest Nusa Tenggara

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reported that the development of the fetal nervous system is of high risk to the toxic effects of mercury. Other systems that may be affected by mercury toxicity are the respiratory, gastrointestinal, hematologic, immune, and reproductive systems. Following exposure to elemental mercury and methylmercury, kidney damage is the end-point of exposure to inorganic mercury compounds. Exposure damages the secretory organ of erythropoietin-a hormone that stimulates erythrocyte synthesis the kidney. As kidney function declines, this affects the amount of red blood cells.<sup>5,11,16,17</sup> In addition, the developing nervous system has been identified as the most sensitive toxicological endpoint.5,11,12,17

Since mercury vapor is easily absorbed into the human body, an assessment of miners and people living in the vicinity of ASGM is urgently needed. However, in Indonesia, the analysis of hair and urine samples as a proof test for total mercury toxicity is expensive, as few laboratories are able to analyze total mercury or even methylmercury. Based on the authors' experience, samples must be sent to a laboratory out of West Nusa Tenggara Province or even overseas. Hence, other tests must be considered to identify the first symptoms of mercury toxicity among miners

ad the surrounding community. Bose-O'Reilly et al. recommended four neuropsychological tests to assess memory and motor function problems in ASGM miners.<sup>18</sup> A physical was aimed at identifying neurologic disturbances such as ataxia, tremor and coordination problems related to the neurotoxic effects of mercury. Furthermore, preliminary symptoms of mercury exposure can be determined by examining kidney function through a simple urine test and hemoglobin level can be assessed with a simple blood test kit. Holmes, et al. reported that there was an increased level of excretion of low molecular weight proteins in urine from ASGM workers of as low as 5-10 µg/g creatinine.<sup>19</sup> These creatinine levels were only slightly above those found in the general population. Even though this level was only slightly higher than normal, the significance of such changes in renal excretory profile is of toxicological importance. Furthermore, mercury ingested or inhaled as elemental mercury binds to structural protein-hemoglobin and causes impairment in the erythrocyte function of oxygen carrying capacity and induces hemolysis. Both mechanisms could cause decreases in either hemoglobin (Hb) or hematocrit (HCT) levels.<sup>20</sup> These findings could be used to take further action for workers and the entire community

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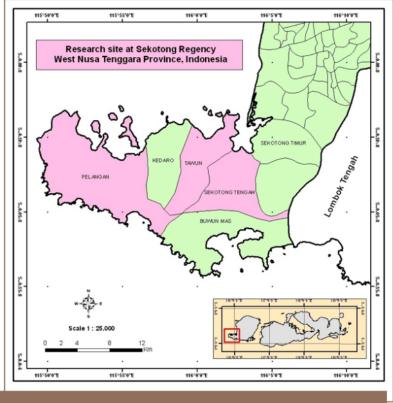


Figure 1 — The study site at Sekotong District West Nusa Tenggara Province

in the vicinity of ASGM. This study aimed to determine the association between mercury exposure, renal function and hematological parameters of gold miners and the surrounding community as a first indication of mercury exposure symptoms.



Study Location The research was conducted at Sekotong ASGM, West Lombok Regency, West Nusa Tenggara

**Province, Indonesia** (*Figure 1*). The subjects were miners and families from 3 different villages: Sekotong Tengah, Tawun and Pelangan. The

villages were the main gates to the mining sites.

#### Sample Collection and Preparation

The samples were recruited using a non-probability sampling technique (purposive sampling) by collecting 33 samples in each village, and in total, 100 people were recruited from the 3 villages. The subject criteria were: 1) miners who had been mining and using mercury for at least 5 years; and 2) non-miners, including ives and children, who had lived in the gold processing area for at least one year. Before conducting the study, ethical clearance was obtained 25 m the Ethical Code Board of the Medical Faculty, University of Mataram, Research

Indonesia, where subjects were asked to sign an informed consent form in order to be involved in this study. Blood and urine samples were then obtained from the miners and non-miners. Following the study procedure, the miners and nonminers were questioned about their mercury exposure over the previous 5 years, duration of exposure, and how mercury was handled in their daily life. Blood and urine samples were collected at the time of the study, around 10 ml of urine and 0.1 ml of blood (214 ops) were collected per subject. In order to determine the parallel results between the bloodurine and hair results, hair from the miners was collected at a different time for analysis.1

#### Sample Analysis

Proteinuria was assessed immediately upon collection, but urine was stored at -4°C until analysis for mercury. Proteinuria level was assessed using dip-stick urinalysis and the results were stated as g/L. The hemoglobin and hematocrit via capillary blood sample were assessed using a hemoglobinometer and stated as mg/dL.

For determining the parallel results of proteinuria and hemoglobin and hematocrit, some of the miners' hair and urine samples were analyzed. Analysis of the hair samples was performed at State Key Laboratory of Environmental Geochemistr 4 in Guiyang, China.<sup>1</sup> Hair samples for total mercury examination were directly assessed using a Lunex RA915+ mercury analyzer. The detection limit of the instrument was 0.2–5 ng g–1. The urine samples were directly collected, and placed and sealed in a polyethylene container and kept in the 15 ezer at -20°C until transport to the laboratory of the Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine in Munich,

Characteristics	Percentage (%)
Sex	
Male	75
Female	25
Age (year)	
<18	10
18-60	85
>60	5
Occupation	
Miner (directly exposed)	71
Non-miner (indirectly exposed)	29
Duration of exposure (years)	
Mean	5.5
<1 (n=0)	0
1-5 (n=54)	53
6-10 (n=4)	47
>10 (n=1)	0

Table 1 — Characteristics of Research Participants

	Level			
Parameter	Minimum	Maximum	Mean ± Standard deviation	Median
Proteinuria	1.0	3.0	2.0	2.0
Level of protein in urine (g/L)	1.0	3.0	1.6±1.0	1.0
Hemoglobin (g/dL)				
Male	7.3	25.0	12.9±2.7	12.7
Female	9.6	16.9	13.1±1.5	13.0
Hematocrit (%)	21.9	75.0	38.9±7.3	38.4
Urine mercury level (µg/L)	2.6	178.1	41.0±52.2	19.3
Hair mercury level (µg/g)	0.0	6.6	2.6±1.7	2.2

 $Table \ 2-Characteristics \ of \ Proteinuria, \ Urine \ Mercury \ and \ Hematological \ Parameters$ 

Germany. The analysis of mercury in urine was performed with cold-vapor atomic absorption spectrometry and the determination limit was 0.25 $\mu g/l.$ <sup>9</sup> Blood and urine data were analyzed using descriptive statistical methods and Spearman's correlation **6** t. It was found that after a short time (4 years) of mercury exposure, the concentration of total Hg in miners' hair and urine from Sekotong ASGM were above the World Health Organization (WHO) standard. <sup>16,8</sup>

### Results

### Characteristic of Research Participants

This research involved one hundred ASGM workers and their families, the characteristics of whom are shown in Table 1.

Table 1 shows that most participants were male (75%), and 90% were adults and 10% were children. Most participants were miners (71%) working in processes such as amalgamation, cyanidation, and smelting. Hence, they were directly exposed to mercury. Non-miners, the family of miners (wives and children), were considered to be indirectly exposed to mercury, as they lived in the vicinity of the smelting process within a radius of less than 500 m. Their duration of exposure was more than five years (mean = 5.4 years).

Data shown in Table 2 are a compilation of all parameters measured in this research, in 27 ding urinalysis, hemoglobin, and total mercury in hair and urine. Protein was assessed qualitatively using the dip-stick method and was categorized as negative, trace, +1, +2, +3, or +4 and quantitatively valued as negative, <0.3 g/L, 0.3 g/L, 1 g/L, 3 g/1 and >20 g/L. The average amount of protein in urine was  $1.56 \pm 1.03$  g/L (mean SD). This was considered to indicate

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Proteinuria (g/L)	Percentage (%)	
Miners		
	0	
<0.3	0	
0.3	7	
	56.3	
	36.3	
≥20	0	
Non-miners		
	0	
<0.3	0	
0.3	27.6	
	48.3	
	24.1	
≥20	0	
Table 3 — Proteinuria Profile in		
Sekotong Area ASGMs		

Hemoglobin Level Group	Percentage (%)	Hematocrit Percentage	Percentage (%)	
Miner		Miner		
<13 g/dL	57.7	<40%	67.6	
≥13 g/dL	42.3	40-50%	28.2	
Smoking		≥50%	4.2	
<13.3 g/dL	67.6			
≥13.3 g/dL	32.4			
		Non-miner		
Non-miner		<40%	51.7	
<12 g/dL	13.8	40-50%	41.4	
≥12 g/dL	86.2	≥50%	6.9	
Table 4 — Hemoglobin and Hematocrit Levels of Subjects in Sekotong Area ASGMs				

proteinuria since this level was higher than 300 mg/L (0.3 g/L). The mean value of hemoglobin found in men (12.9  $\pm$  2.68 g/dL) was lower than in women (13.11  $\pm$  1.45 g/dL). The mean hematocrit value was 38.95  $\pm$  7.27%, and mercury levels in urine and hair were 41.04  $\pm$  52.19 µg/L and 2.56  $\pm$ 1.71 (µg/g), respectively.

Table 4 shows that 57.7% of the miners suffered from anemia, as the cutoff point for this value was 13 g/dL among male non-smokers. However, if the cut-off value for anemia among smokers is taken into consideration (13.3 g/dL), 67.6% of the miners would be considered anemic. The results also showed that the Hb values of nonminers were predominantly normal compared to miners, as only 13.8% of non-miners suffered from anemia.

Table 5 illustrates the correlations between mercury exposure and several health parameters such as urine protein level, Hb, HCT, urine and hair mercury levels in two different mercury exposure groups, miners and non-mir23s. Spearman's correlation test and the Kruskal-Wallis test were performed to confirm the relationships between the miners and non-miners and health parameters. Table 5 shows that miners and non-miners showed significant digrences in all health parameters (p <0.05 Kruskal-Wallis test and ρ <0.05 Spearman's terzeexcept for hair mercury value, which showed no significant difference between the miners and non-miners.

### Discussion

All participants in the present study were directly exposed to mercury, either as workers (miners) or as family members (non-miners) living in the contaminated atmosphere, water, food stuff and soil in the vicinity of the miner activities. Most participants were directly exposed to mercury, as they worked in the cyanidation process, since this process often used sludge from the amalgamation method which involves mercury.

Some study subjects were children that had direct contact with mercury from mercury panning after school and standing in close proximity to burning processes. This type of child activity has also been reported in other gold mining areas.9 Most of the women were of reproductive age, which means that their pregnancies were also at risk, as mercury affects intrauterine growth development, 10 ecially brain development.5,21,22 The duration opexposure to mercury contaminants was an average of 5.4 years. This period was much shorter than the 14.8 years needed to show

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Exposure Group	Urine protein level (g/L)	Hemoglobin (g/dL)	Hematocrit (%)	Urine Mercury Level (µg/L)	Hair Mercury Leve (µg/g)
Miners	$1.68 \pm 1.023$	12.74±2.39	38.21±7.18	69.39±62.41	2.77±1.68
Non-miners	1.29±1.03	13.59±2.43	40.77±7.29	12.7±11.50	2.37±1.82
Spearman's rho (ρ); coefficient correlation	0.031;-0.216	0.045; 0.201	0.045; 0.201	0.042; -0.550	0.517;-0.169
Kruskal-Wallis Test	0.032	0.045	0.045	0.047	0.500

specific clinical manifestations in previous reports.<sup>11</sup> This manifestation is affected by the type and dose of mercury, the age or developmental stage, duration of exposure and route of exposure.<sup>5</sup> A weakness of the present study was that there was no identification of the dose and type of mercury exposure.

Protein levels in urine were obtained by converting the qualitative proteinuria dip-stick results categorized as negative, trace, +1, +2, +3, 11 to quantitative values of 0 g/L, <0.3 g/L, 0.3 g/L, 1 g/L and ≥20 g/L, respectively. Proteinuria was identified by results of +2 and  $\geq$ 0.3 g/L.<sup>21</sup> Almost all of the subjects in the exposed areas, both miners and non-miners, were positive for proteinuria (miners, 92.6% and non-miners, 72.4%). This result was similar to findings of proteinuria in patients exposed to products ntaining mercury.<sup>24</sup> In addition, proteinuria is a clinical manifestation of mercury intoxication with elemental, inorganic and ethylmercury.<sup>17</sup> High urinary (69.39 ± 62.41 1/L) and hair (2.77 ± 1.68  $\mu g/g$ ) mercury levels in the miners indicate that a pathological process in the kidney may occur due to mercury exposure. Common causes of urinary

mercury excretion were elemental and organic mercury 30 posure, as miners were directly exposed to mercury vapor during the 111elting process.<sup>10,25</sup> Once inhaled, mercury vapor is dispersed rapidly into the blood and might deposit in other organs e.g. brain, kidney, placenta thyroid and others.7,11 Significant differences in urinary levels between miners and non-miners indicate that a possible route of exposure may be ingestion of inorganic mercury though contaminated food or water.1 In the study location, most of the water sources for amalgamation processes and daily life come from the same well, and often the tailing ponds are nearby the well. Although both groups were directly exposed to mercury, the level of mercury (urinary and hair samples) for the miners group was higher than for non-miners. One contributing factor in this case was smoking habit, as all miners were smokers, and the urinary mercury level of this group was more than five times higher than that of non-miners.13

Persistent proteinuria indicates kidney disease and the most common impacts of this condition are diabetes, hypertension, obesity and medicine or chemical substances.<sup>26</sup> One of the chemical substances that affects proteinuria is mercury, and to confirm this, repeated tests are required.<sup>25</sup> People with consistent positive results can be categorized as having persistent proteinuria and considered to have kidney disease. A weakness of the present study was that there were no repeated tests to confirm persistent proteinuria. Assessment of renal function is needed in future studies.

The mean of the hemoglobin concentration of miners  $(12.74 \pm 2.39)$ g/dL) was lower than that of either non-miners  $(13.59 \pm 2.43 \text{ g/dL})$  or the normal value for men (13-18 g/dL).6 T<sub>11</sub> results of this study also indicated a correlation between the smoking habits of miners and hemoglobin concentration. Since all of the miners were smokers, if a correction factor for smokers (0.3) is used, then Hb values would appear normal (13.04 However, when the normal level for male smokers is used (13.3 d/dL), then the Hb values of the miners were below normal.

Hemoglobin concentration is affected by many factors such as high altitude, diet, pregnancy and smoking.<sup>27</sup> Since the Sekotong mining sites are in the lowlands, subjects were not pregnant

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and had a homogenous diet, the remaining factors influencing Hb values were smoking and mercury exposure (route and magnitude). This finding was similar to that of previous studies reporting that mercury exposure could decrease hemoglobin levels.<sup>20,28</sup> The mechanisms behind the decrease in Hb through mercury exposure are not only due to decreasing renal function, but also due to the influence on iron metabolism and haemolysis.16,29,30 To examine the iron metabolism factor. iron transfer and ferritin serum must be assessed, which was not performed in the present study. The Hb values and HCT concentration of miners  $(38.21 \pm 7.18\%)$  were lower than that of non-miners (40.77 ± 7.29%) as well as the normal value (40-50%). Hematocrit reflects the cell and plasma proportions, and low Hb is usually found in low HCT and vice versa.

Except for hair mercury level, all parameters m24 rred in the present study showed significant differences between the two groups. The subjects in the miner group were men, and most subjects in the 28 n-miner group were women. The results of the present study differed from those of previous reports that found that mercury in men's hair was higher than that in women's.<sup>24</sup>

### Conclusion

After five years of exposure to mercury, people in Sekotong area ASGMs, both morers and nonminers, showed proteinuria and low hemoglobin and hematocrit concentrations due to chronic Percury intoxication as indicated by high urinary and hair mercury levels.

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