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Silicosis and its Progress Influenced by Genetic Variation on TNF-α Locus- 308, TNF-α and IL-10 Cytokine on Cement Factory Workers in Indonesia

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Abstract: This study aimed to assess the association of Tumor Necrosis Factor (TNF-α) Locus-308 variant, TNF-α and Interleukin (IL-10) Cytokine with the risk of silicosis and its progress in Indonesian cement factory workers, There is an urgent need to explore the determining factors other than exposure since silicosis is chronic progressive and life threatening but remains found, though much industrial hygiene effort has been made. This study population was 6,069 workers registered during 31 December 1990 to 31 December 2003. First, prospective study with Nested Case Control design was conducted on 336 workers in 2003, ten years later the progression of silicosis was assessed in 2013. The result showed proportion of the genetic variation on TNF-α on Locus -308 in Indonesia was significantly (p = 0.02) higher on silicosis (13.45%) than nonsilicosis (5.45%) but lower than silicosis in Africa and US miners, since susceptibility loci might vary in different ethnic groups. The sign and symptoms remained as simple silicosis after ten years; The TNF-α:IL-10 ratio >1 was a risk factor in silicosis; the ratio of TNF-α:IL-10 >1 caused a rapid decline of lung function compare to ratio <1, the decline was chronic progressive during ten years yet not causing significant dyspnea among the cases. Further studies with enlarged sample size are needed. The study concluded, the genetic variation on TNF-α gene locus -308 was a risk factor of silicosis in Indonesia cement factory. Its role is indirect, but through mechanism of controlling the blood Cytokine level ratio of TNF-α toward IL-10.

Key words: Genetic variation, TNF-α, IL-10, cement, silicosis

INTRODUCTION

Silicosis is a life threatening occupational disease but remains widespread though much industrial hygiene effort has been made. International Labour Organization (ILO) recently reported that primary industries and high-risk sectors in developing countries are at risk of pneumoconioses, silicosis is the prominent one. The most prevalence was stated in China, India, Vietnam and Brazil (International Labour Organization, 2013). Silicosis likes other pneumoconioses, the nature of the disease is chronic progressive beyond the time of exposure, even found on retirees in the developed countries that had stopped the mining and had been implementing industrial hygiene (Corbett *et al*., 2002). Cement industry is a mining industry, workers are exposed to silica compound or free silica since silica is a common mineral that is part of sand, rock and mineral ores.

Silicosis is the prominent of pneumoconioses. The current classic epidemiological research has yet to answer the question of why those workers with similar dose and length of exposure will not result in the same outcome. There is an urgent need to explore the determining factors other than exposure. With the finalization of human genome project and its objective to map all the genes that construct the structure and functions of human cells has opened a new horizon of various genetic factors, that play role in human response toward environmental exposure (Yucesoy *et al*., 2001; Corbett *et al*., 2002; Morimoto and Tanaka, 2001). Peripheral blood TNF-α cytokine level was a very useful parameter in diagnosing early stage of silicosis (Miao *et al*., 2011). It is known that factors determining TNF-α cytokine production stimulated by chronic inflammation is the genetic variation on TNF-α locus -308. The other factor for the inflammation process due to mineral dust is the IL-10 concentration and IL-10 is the anti-inflammation cytokine secreted after the inflammation (Mossman and Churg, 1998; Kunkel and Strieter, 1998; Strieter, 2001). Furthermore, TNF-α gene promoter is known may predispose to severe silicosis (Corbett *et al*., 2002). Through the genetic and molecular epidemiology approach, it is expected to comprehensively understand how silicosis occurs and develops chronic progressively. Furthermore, with better pathogenesis understanding, an intervention action can be taken for prevention.

All the cement production process starting from the mining of raw materials, milling and combustion until the

packaging of end-product, in the form of clinker or cement meal, is related to mineral particles from bulky rocks to microscopic event nano-particle dust, the raw materials of cement originated from earth rocks, especially from lime stone and clay, which contain silica in all form. Cement is produced almost everywhere in the world. Cement industry has been established for almost a century in Indonesia and is one of strategic industries supporting the national development (Asia Federation of Cement Manufactures, 2000). Up to recently, there is view information of the silicosis evidence among the cement workers. Uncertainty is a less conducive precondition toward the preventive and protective effort for the workers.

The aim of this study is to identify the role of genetic factors contributed to the occurrence of silicosis among cement factory workers. This study analyzes the immunogenetics factor in silicosis occurrence and its progress among cement factory workers, by examining the genetic variation on TNF-α gene locus -308 and the interaction dynamics between TNF-α as a pro-inflammatory cytokine and the IL-10 as an anti-inflammatory cytokine.

MATERIALS AND METHODS

This study population was 6,069 workers who are registered in a cement factory during 31 December 1990 to 31 December 2003; 336 of 6,069 workers were invited in 2003 to participate as respondents. The blood used for laboratory DNA gen testing was collected from the respondents.

The cumulative incidence and incidence density were adapted from the whole population. The role of genetic variation to the occurrence of silicosis and its progress had been investigated through a Nested Case Control study, a design of prospective study, among 336 workers. After ten years follow up, the progression of silicosis was assessed in 2013.

Diagnosis methods, equipment used and chest X-ray performed were in accordance with the ILO standard (International Labour Office, 1980). Silicosis was diagnosed if fulfilling the criteria of category 0/1 or more. Information on respiratory symptoms, smoking habits and compliance to use Personal Protective Equipment (PPE) summarized from a survey using modified American Thoracic Society standard questionnaire (Ferris, 1978). Lung function testing performed according to American Thoracic Society spirometry standard procedure (Horvath and Frostman, 1980). An Indonesian lung function standard published by Pnuemobile® Project (Alsagaff and Mangunnegoro, 1993) had been used as comparison. Other demographic information related with employment adapted from the company personnel database. Laboratory DNA gene testing was carried out under the PCR-RFLP technique, using *Nco*I enzyme to identify the genetic variation on TNF-α gene locus -308. TNF-α and IL-10 level were measured under ELISA method. The dependent variable in this study was the time frame from the first day of employment in cement factory until the first time silicosis was diagnosed (event). The independent variable was the major risk factors i.e., the genetic variation on TNF-α gene locus -308, the free silica contained dust cumulative exposure and other risk factors such as age when entering the workforce, smoking habits and use of PPE. Statistical analysis was conducted with a computer software Stata. Silicosis's risk factors assessment and scoring model development are conducted by survival analysis and Cox regression multivariate analysis.

RESULTS AND DISCUSSION

This study concluded that silicosis has been found among cement factory workers in Indonesia. Abnormal chest X-ray in the form of stellate were found in a greater number in this study; an evidence to the possible occurrence of mixed dust pneumoconiosis. During the period of 13 years, the cumulative incidence is 2.90% (category ≥0/1) and the incidence density is 16 cases per 10,000 every year. When using category 1/0 or more, the cumulative incidence became 2.06% (Table 1). Previous the study, the incidence data of silicosis did not exist in Indonesia. Silicosis (category ≥1/0) prevalence found among 26 factories out of 153 cement factories operated in the US is 1.89% (Abrons *et al*., 1997). Those finding supported the fact that silicosis among cement factory workers is rare. As the disease is chronic progressive, it may affect the quality of life. A surveillance on silicosis among the cement factory workers and working environment risk assessment should be carried out. A prompt continuous improvement should be established. The cement factory management should implement an occupational health program such as industrial hygiene, regular medical examination and health promotion. The recommendations made in 2003 were carry out in this

Table 1: Silicosis cumulative incidence among cement factory workers (January 1, 1991 -December 31, 2003)

	Case (n = 6069)	
Silicosis categories	N	%
0/1	51	0.840
1/0	1	0.016
1/1	124	2.043
Total	176	2.899

Table 2: Distribution of genetic variation on TNF-α gene locus -308 in silicosis and nonsilicosis

	Silicosis		Nonsilicosis		
Prime risk factor	N	%	N	%	p-value
TNF-α genotype locus-308 (n = 336)	-	-	-	-	0.020*
GG	148	86.55	156	94.55	
AG	21	12.28	9	5.45	
AA	2	1.17	0	0.00	

*Fisher's exact

factory, after ten years follow up (until March 2013), there was no new case emerged and none of case was getting worse but 17 of them were passed away and none of them was recorded as pulmonary death.

The silicosis cases under 23 years study were expected to develop into simple chronic bronchitis. The symptoms of this disease is chronic coughing with phlegm without shortness of breath, precede the chronic obstructive pulmonary disease. Coughing triggered by irritated nodes, aggravated the production of phlegm and hypersecretion; the finding supported other cement factory workers researchers overseas. Analysis after excluding smoking habits from the data, risks of chronic bronchitis among those with silicosis and no silicosis were not significantly different. The discrepancy should be monitored, as the simple chronic bronchitis might be followed by chronic obstructive pulmonary disease. Further investigation should be carried out whether there is a possibility of confounding or interaction with smoking habit, another risk factors or other diseases that may cause chronic bronchitis.

Silicosis in the cement factory were found without shortness of breath. This silicosis was a type of chronic silicosis or simple silicosis. This type of silicosis is not occupational health problem, as it has less clinical symptoms. This study had proved that some may live for a long time until pass away without significant symptoms. Although silicosis had no symptoms, the management should removed those workers with the disease from the exposure. They have to perform health promotion in the workplace and establish general occupational health program. After ten years implemented these programs, all workers who were successfully protected from the silicosis and its complications. Those programs should be conducted consistently.

There was a significant different proportion of the genetic variation on TNF-α gene (-308) distribution between silicosis and nonsilicosis workers (p = 0.032). AG genotype was found more prevalent among workers with silicosis (12.28%) compared with nonsilicosis (5.45%), whereas AA genotype was only found among workers with silicosis (1.17%) (Table 2). Further analysis showed that the proportion of genetic variation on TNF-α gene locus -308 among workers with silicosis was 13.45% but workers without silicosis was only 5.45%, significantly different (p = 0.02). The finding of this study was in line with other studies in both coal mining and underground gold mining. However, the proportion of genetic variation on TNF-α gene locus -308 among workers with silicosis in Indonesia was smaller compared with the similar genetic variation among workers with silicosis in South Africa coal mining (39.67%) and underground American gold mining (58.16%) (Corbett *et al*., 2002). The differences were thought due to racial different of the study population: Asian, Black and Caucasian. The possibility of the genotype different has a role in the phenotype manifestation of silicosis needs further study.

The mechanism of silicosis occurrence in cement factory is evidently very complex; there is an interaction among genetic variation, immune system and environmental factors. We found that workers with genetic variation on TNF-α gene locus -308 had higher risks to get silicosis/pneumoconiosis than those without that genetic variation. This findings supported by the fact that those workers with genetic variation (75%) produce more TNF-α cytokine than those without that genetic variation (48.03%), significantly different (p = 0.004) (Table 3). The higher production of TNF-α cytokine was thought due to chronic exposure to the inflammatory agent coming from cement factory environment, such as mineral dust from the earth rock, clinker or cement. TNF-α is known as proinflammatory and profibrosis cytokine.

The inflammation process will stimulate the IL-10 production as the anti-inflammatory cytokine to balance them (Mossman and Churg, 1998; Kunkel and Strieter, 1998; Strieter, 2001). IL-10 level, which is anti-inflammatory and profibrosis, increased among workers with silicosis. If the ratio TNF-α:IL-10 is less than one, the anti-inflammatory effect of IL-10 is dominant and reduced the inflammatory effect of TNF-α. In that case, inflammation does not occur and the fibrosis process does not occur. Thus, the silicosis does not occur. In contrary, if the ratio of TNF-α:IL-10 is more than one, anti-inflammatory effect of IL-10 is unable to suppress TNF-α proinflammatory effect, as shown there was significant difference (p = 0.009) between the ratio of TNF-α toward IL-10 more than one compare to less than one, regardless with or without genetic variation on TNF-α (-308), it were 100 and 77.70%, respectively (Table 3). Fibrosis process is continued and results in workers with silicosis. Lung function parameters of the workers will diminish and workers complain respiratory problem. But it was not the case, in this study, not even one case showed the decrement of lung function (FEV1 and FVC) was bigger than the decrement of lung

Table 3: Distribution of tnf-α concentration, il-10 concentration and tnf-α:il-10 ratio

	All Subject (n = 342)				Silicosis and Nonsilicosis (n = 336)				Silicosis (n = 171)			
	Nonsilicosis		Silicosis		With no gen.variation		With gen. variation		With no gen. variation		With gen. variation	
Cytokine	n	%	n	%	n	%	n	%	n	%	n	%
TNF-α concentration												
<43.192 ρg mL^{-1}	161	96.99	6	3.41	158	51.97	8	25.00	6	4.05	0	0.00
≥43.192 ρg mL^{-1}	5	3.01	170	96.59	146	48.03	24	75.00	142	95.95	23	100.00
p-value	0.000				0.004				0.100*			
IL-10 concentration												
<45.614 ρg mL^{-1}	152	91.57	10	5.68	148	48.68	13	40.63	3	2.03	7	30.43
≥45.614 ρg mL^{-1}	14	8.43	166	94.32	156	51.32	19	59.38	145	97.97	16	69.57
p-value	0.000				0.385				0.000*			
TNF-α:IL-10 ratio												
<1	92	55.42	38	21.59	120	39.47	4	12.50	33	22.30	0	0.00
>1	74	44.58	138	78.41	184	60.53	28	87.50	115	77.70	23	100.00
p-value	0.000				0.002*				0.009*			

*Fisher's exact

Table 4: Predictive score of the occurrence of silicosis among cement factory workers

Risk factors	Score (s)	Subject characteristics (I)	Score S×1
TNF-α:IL-10 Ratio	3		
Age Starting Employment			
<31 years old	0		
≥31 years old	1		
Total score			

Silicosis is predicted if the score is ≥3.30

function among normal population. The decrement of lung function related solely with the ageing process, it is the nature of lung function.

Survival analysis showed that more cement factory workers with genetic variation on TNF-α gene locus -308 developed silicosis compared with those without the genetic variation. A ten-year survival on silicosis workers without genetic variation was higher than those with genetic variation. This finding supported the hypothesis, where genetic variation on TNF-α gene locus -308 plays an important role in occurrence of silicosis or pneumoconiosis among the cement factory workers. However, the role is indirect, but through the control mechanism of TNF-α:IL-10 ratio. This was proved by the next ten years' results, all the silicosis cases remained as simple silicosis, meaning that IL-10 could diminish the inflammatory effect of TNF-α cytokine.

It was found also the major risk factor of the silicosis occurrence is the ratio of TNF-α:IL-10 and the age entering the workforce. For practical use, a scoring form was designed for user-friendliness. The grouping of data scoring was conducted through evaluating all the silicosis cases and a study was conducted to check for its sensitivity and specificity, so that we obtain the scoring and grouping from prediction analysis of silicosis cases that were scored with two variables as risk factors, namely the ratio of TNF-α:IL-10 and age at the first date of employment in the cement factory. The scoring form shown in Table 4.

CONCLUSION

The genetic variation on TNF-α gene locus -308 was found more frequent among the silicosis subject than nonsilicosis. In the cement factory, there were more workers with the genetic variation on TNF-α gene locus -308 who develop into silicosis compared with subject without the genetic variation. A ten-year survival of silicosis subject without the genetic variation was greater than the silicosis subject with the genetic variation. This finding was the hypothesis to be proven, that was the genetic variation on TNF-α gene locus -308 plays a significant role in silicosis/pneumoconiosis occurrence in cement factory. Its role is indirect but through mechanism of controlling the ratio of TNF-α toward IL-10.

The ratio of TNF-α:IL-10 <1 means protective, >1 is a risk factor for silicosis. This finding supported the hypothesis, where the ratio of TNF-α:IL-10 is the silicosis risk factor.

The role of the genetic variation on TNF-α gene locus-308 is indirectly related with the clinical manifestation and lung function of silicosis patient. The clinical symptoms are a complex phenomenon of the more complex phenotype. Phenotype is an interaction between environmental factors and genetic factor and between one gene with another that has role in the workers response to the long lasting external stimuli.

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