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The Effects of Hazardous Chemical Exposure on Cardiovascular Disease in Chemical Products Manufacturing Workers

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Abstract

The purpose of this study was to understand the mechanism of cardiovascular disease (CVD) caused by exposure to hazardous chemicals. We investigated changes in the symptoms of metabolic syndrome, which is strongly related to CVD, and in levels of other CVD risk factors, with a special emphasis on the roles of catecholamines and oxidative stress. The results revealed that neither body mass index (BMI) nor waist and hip circumferences were associated with exposure to hazardous chemicals. Among metabolic syndrome criteria, only HDL-cholesterol level increased on exposure to hazardous chemicals. Levels of epinephrine (EP) and norepinephrine (NEP) were not influenced by exposure to hazardous chemicals; however, the total antioxidative capacity (TAC) reduced because of increased oxidative stress. Both hazardous chemical exposure level and metabolite excretion were related to EP, NEP, and the oxidative stress index (OSI). Logistic regression analysis with these factors as independent variables and metabolic syndrome criteria as dependent variables revealed that EP was associated with blood pressure, and NEP with metabolic syndrome in the chemicalexposed group. In conclusion, the results suggest that reactive oxygen species generated and oxidative stress due to exposure to hazardous chemicals act as mediators and cause changes in the physiological levels of EP and NEP to increase blood pressure. T mately leads to the development of CVD through increase in cholesterol, triglyceride, and glucose levels by lipid peroxidation.

Keywords: Cardiovascular disease, Catecholamine, Hazardous chemical, Workers

INTRODUCTION

Although in some developing countries mortality by cardiovascular disease (CVD) shows decreasing trend, worldwide - including Korea - mortality by CVD is continuously increasing (KMHW, 2011; Rosamond *et al*., 2008). Many epidemiological studies have been conducted in order to fundamentally understand the present situation and to predict and settle the future issues, and their results show that development of CVD is due to bad life style, and bad life style is related with changes in industry structure and economic growth (Jung *et al*., 2007; Kim and Chun, 2009). Modern society requires more mental activity rather than physical one at both home and workplace due to the change to the industrial structure pursuing progress of science and high-added values based on knowledge economy, and this trend leads to increased CVD risk through under- or overnutrition caused by body energy imbalance (Redman *et al*., 2008). Another cause suggested for increased CVD risk is decreased function of nervous-endocrine system and circulatory system due to imbalanced internal homeostasis regulators (Lebrun *et al*., 2006). As mentioned above, CVD is induced by a variety of factors, and the mechanism of induction has not been clarified by etiological studies for each factor. However, CVD is thought to be induced by interaction between modifiable risk factors (MRF) such as life style and environmental factors and non-modifiable risk factors (NMRF) such as gender, age and genetic factors (Hong *et al*., 1997). In epidemiological study conducted in 2,248 CVD patients, Baena *et al*. (2005) reported that 35.2% of total subjects were smokers, and 33.7%, 21.9%, 12.7%, and 15.8% had hypertension, hypercholesterolemia, hyperlipidemia, and diabetes, respectively; 57.9% of total subjects had at least one CVD risk factors. Other studies observed the association between hypertension and family history (Wu *et al*., 2008; Choi *et al*., 2009). The observation is presumed to result from changes in physiological levels of proteins which are involved in blood pressure regulation from the differences in life style of parents, i.e. differences in food intake (Al-Safi *et al*., 2006), and expression of specific genes, which may influence CVD development from childhood (Periaswamy *et al*., 2008; Czupryniak *et al*., 2008). In a study in Japanese workers, Yamada *et al*. (2007) reported that genetic polymorphisms of insulin receptor substrate 1 (IRS1) and β3-adrenergic receptor (β3-AR) were associated with CVD risk factors such as obesity, insulin resistance and type 2 diabetes development; Kim *et al*. (2009) also reported that obesity was sensitive to IRS1 genetic polymorphism in Korean workers. Besides, many researches are being performed for various genes involved in progression of obesity (Clément, 2006). In addition to MRF and NMRF mentioned above there are a variety of CVD risk factors which come from personal life and working environment and directly/indirectly influence other risk factors to accelerate the development and progression of CVD, among which the most prominent one is stress. Stress affects CVD development through increased desire for a cigarette of smokers and quantitative change in food intake (Mitchell and Perkins, 1998), and acts as an inducing factor for obesity, hypercholesterolemia and hyperlipidemia by changing the physiological levels of neurotransmitters and neurotrophics which are involved in feedback regulation of CVD risk factors (Smith, 1996; Mitoma *et al*., 2008). In this way MRF and NMRF not only directly affect specific organs, but also affect CVD development through changing the physiological levels of CVD risk factors by influencing the neuropeptides, neurotransmitters and cytokines (Lebrun *et al*., 2006). Maintaining the levels of internal homeostasis regulators is crucial to optimizing the response to external stress and biofunction, and they are directly involved in development and progression of various diseases including CVD. Imbalance of homeostasis regulators is highly associated with exposure to environmental pollutants as well as life style (Albright and Goldstein, 1996),

and the extent of imbalance depends on the exposure level (Merker *et al.*, 2006). However, previous studies on the exposure to hazardous chemicals are mostly studies on target organ toxicity based on pharmacokinetic investigation, enzymological studies, and studies by specific area, e.g. neurotransmitters and immune system, and there are very few studies directly addressing the relation with CVD.

Several researchers reported that exposure to hazardous chemicals causes high CVD occurrence by elevating the physiological levels of brain-derived neurotrophic factor (BDNF), leptin and neurotransmitters, i.e. catecholamines, serotonin and dopamine which are related with desire for food intake (Gurley *et al.*, 2007; Monroe and Balvorsen, 2009), and that exposure to SO_2, CO_2, NO_2 and PM_{10} from environmental pollution also influence CVD development (Goncalves *et al.*, 2007). Kotseva and Popov (1998) reported that workers occupationally exposed to high-level hazardous chemicals in a petrochemical factory had an increased prevalence of hypertension, and Seppalainene and Harkonene (Seppalainen and Harkonen, 1976) also reported that workers exposed to high-level styrene had higher prevalence of cardiovascular disorder than workers with lowlevel styrene exposure or normal population. Heo *et al.* (2009) reported that CVD risk factors such as systolic blood pressure, cholesterol and fasting glucose were higher in workers chronically exposed to high-level styrene in plastic product manufacturing factories than in non-exposed controls. Although several authors have reported the association between exposure to hazardous chemicals and CVD risk factors as mentioned above, there have been very few studies on the process and endogenous substances involved therein.

The purpose of this study was to understand the mechanism of CVD developing by hazardous chemicals through investigating the changes in criteria for metabolic syndrome which is highly related with CVD and in levels of other CVD risk factors by exposure to hazardous chemicals, and the roles of catecholamine and oxidative stress during the process of changes.

MATERIALS AND METHODS

Study subjects. Exposed group was 180 male workers who had been chronically (for more than 10 months) exposed to mixture of organic solvents including styrene, toluene and xylene in plastic product and paint manufacturing factories, and control group was comprised of 135 male workers in machinery and metal product manufacturing factories who had not been exposed to those organic solvents or other hazardous chemicals. Institutional Review Board of Occupational Safety and Health Research Institute, Korea Occupational Safety and Health Agency (OSHRI, KOSHA) reviewed and approved the study proposal. The investigator visited the selected workplaces, explained to workers the objective and method, privacy policy, and other details and precautions of the study, and obtained written informed consent from workers who volunteered to participate in the study.

Questionnaire survey for general characteristics and job characteristics. General characteristics (e.g., age, smoking and drinking habits, regular exercise) and job characteristics (e.g., working duration, working hours per day) of study subjects were examined using self-administered questionnaire and face-to-face interview, and eating habit was examined using the method previously described by this author (Heo *et al.*, 2009).

Measurement of CVD risk factors. The CVD risk factors were measured with NCEP-ATP III Asian criteria (2002), which is a metabolic syndrome criteria closely related to CVD development. Metabolic syndrome was identified by the presence of three or more of the five components listed below (NCEP, 2002):

i) Abdominal obesity: waist circumference ≥ 90 cm for men, ≥ 88 cm for women

ii) Hypertension: systolic/diastolic blood pressure ≥ 130/ 85 mmHg

iii) High-density lipoprotein (HDL) cholesterol: ≤ 40 mg/ dl for men, ≤ 50 mg/dl for women

iv) Hypertriglyceridemia: triglyceride ≥ 150 mg/dl

v) High blood glucose: fasting glucose ≥ 110 mg/dl

Among risk factors, body mass index (BMI) was evaluated by measuring height and weight of study subject using body composition analyzer (X-SCAN plus II, Jawon Medical, Seoul, Korea), and systolic and diastolic blood pressures were measured using mercury manometer after 10-minute rest.

Serum biochemistry test and catecholamines measurement. Blood was collected at 09:00-10:00 AM from workers in fasting state since 10:00 PM previous day. Separated serum was transported to laboratory as frozen. Serum biochemistry tests for ALP, ALT, AST, bilirubin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, GGT, glucose, and HbA1C were performed using automatic biochemistry analyzer (COBAS Integra 400, Roche Diagnostics Ltd., Rotkreuz, Switzerland). Serum insulin was measured by automatic chemiluminescence immune analyzer (Access, Sanofi Diagnostics Pasteur, Inc., Minnesota, USA), and then homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the equation [HOMA-IR= (fastinginsulin(μU/ml)×fastingglucose(mM/L)/22.5]. Epinephrine (EP) and norepinephrine (NEP) were measured with competitive ELISA kit (LDN, Nordhorn, Germany) and microplate reader (Tecan, Salzburg, Austria) at 450 nm and 630 nm according to the method suggested by kit manufacturer, following acylation after extraction from plasma using plate coated with boronate affinity gel.

Measurement of oxidative stress index. Total oxidative status (TOS) and total antioxidative capacity (TAC) were measured using colorimetric test kit (Immunodiagnostik AG, Bensheim, Germany). TOS level was calculated by correction by reference material presented with H_2O_2-equivalent (μM/L) following the measurement of total lipid peroxides in plasma which have direct correlation with oxygen radical. TAC was measured with checking the reactivity of antioxidative substances by determining the level of H_2O_2 remaining after reaction with antioxidative substances in plasma for a fixed time, and was expressed in H_2O_2-equivalent (μM/L). Oxidative stress index (OSI) is a ratio of TOS and TAC, and is calculated using the equation [OSI (Arbitrary Unit) = TOS/TAC].

Measurement of ambient hazardous chemicals and their urinary metabolites. Organic solvents including styrene, toluene and xylene in workplace air were sampled and analyzed using low volume active sampler (Low Flow Sampler 113 D, Gilian Ltd., Florida, USA) according to 'Method 1501: Aromatic Hydrocarbons', a method recommended by National Institute for Occupational Safety and Health (NIOSH, 1996). Organic solvents in air were collected for more than 6 hours with active charcoals as sorbent and sampler flow rate controlled at 200 ml/min. Securely packed active charcoal tubes with sampled organic solvents were shipped refrigerated to laboratory. Sorbent was placed in GC vial, and sample for GC injection was prepared by adding 1 ml of carbon disulfide and agitating for 60 minutes, and was analyzed using gas chromatograph (CP-3800 GC/FID, Varian Ltd., Middelburg, Netherland). Metabolites of each organic solvent were analyzed in workers' urine sample using gas chromatograph (CP-3800 GC/FID, Varian Ltd., Middelburg, Netherland) by the method described by de Carvalho *et al*. (de Carvalho *et al*., 1991).

Statistical analysis. All the data were analyzed using Version 12.0 SPSS statistics program (SPSS Inc., Chicago, USA). Independent t-test was used for comparison between control group and exposed group, and Pearson's correlation coefficient was used for correlations among each variable. For correlation analysis between CVD risk factors and exposure to hazardous chemicals, multiple logistic regression analysis was performed with NCEP-ATP III criteria as dependent variables and epinephrine, norepinephrine and oxidative stress indices as independent variables. All the data are expressed as mean and standard deviation.

RESULTS

General characteristics and levels of CVD risk factors of study subjects. As shown in Table 1, mean age, 38.4 years in control group and 37.4 years in exposed group, was not significantly different between groups. Among job characteristics, mean working durations of control group and exposed group, 111.7 months and 125.7 months, respectively, were not different between groups. However, mean working hours per day of control group, 9.8 hours, was significantly longer (p = 0.001) than that of exposed group, 9.0 hours. General characteristics of study subjects and levels of NCEP-ATP III metabolic syndrome criteria are shown in Table 1. 63.9% (115 subjects) of exposed group were smokers, being higher than control group (43.7%) with statistical significance. Mean number of cigarettes per day was also larger in exposed group. Drinking habit, mean sleeping hours per day and regular exercise did not show difference between groups. Anthropometric values were higher with statistical significance in control group than exposed group, with BMI of 23.8 kg/m^2, waist circumference and hip circumference 84.6 cm and 96.4 cm, respectively in control group, and 23.1 kg/m^2, 82.6 cm and 94.2 cm, respectively in exposed group. Among CVD risk factors only HDL-cholesterol was significantly higher (p = 0.010) in exposed group than in control group, 51.1 mg/dl in exposed group and 47.7 mg/dl in control group. Others including systolic and diastolic blood pressure, total cholesterol, triglyceride and fasting glucose did not show significant difference between groups.

Table 1.

General characteristics of study subjects

Variables	Control (n = 135)	Exposed (n = 180)	*p*-value
Age (yrs)	38.4 (9.3)	37.4 (7.9)	NS
Working duration (months)	111.7 (89.9)	125.7 (86.5)	NS
Working hours per day	9.8 (1.6)	9.0 (1.4)	.001
Smoking habit			.001
Non-smoking, %	76 (56.3)	65 (36.1)	
Current smoking, %	59 (43.7)	115 (63.9)	
Cigarettes per day	9.1 (9.2)	10.7 (7.5)	
Drinking habit			NS
Non-drinking, %	21 (15.6)	28 (15.6)	
Current drinking, %	134 (84.4)	152 (84.4)	
Alcohol consumption (g/week)	145.6 (169.8)	145.2 (134.2)	
Regular exercise, %			NS
Yes	77 (57.0)	93 (51.7)	
No	58 (43.0)	87 (48.3)	
Sleeping hours (per day)	6.5 (1.0)	6.6 (0.8)	NS
Body mass index (kg/m^2)	23.8 (2.8)	23.1 (2.7)	.037
Waist circumference (cm)	84.6 (8.1)	82.6 (7.4)	.028
Hip circumference (cm)	96.4 (5.3)	94.2 (5.3)	.001
Blood pressure (mmHg)			
Systolic	127.1 (12.3)	127.8 (14.8)	NS
Diastolic	76.1 (9.0)	75.1 (10.4)	NS
Total cholesterol (mg/dl)	186.4 (33.9)	190.4 (33.3)	NS
HDL-cholesterol (mg/dl)	47.7 (12.3)	51.1 (10.5)	.010
LDL-cholesterol (mg/dl)	113.6 (29.1)	109.0 (30.4)	NS
Triglyceride (mg/dl)	171.1 (118.3)	184.5 (157.8)	NS
Blood glucose (mg/dl)	91.5 (22.4)	91.6 (16.6)	NS

NS: non significant.

Concentration of ambient hazardous chemicals and their metabolites in exposed workers. Subjects in exposed group were under exposure to organic solvents mixture of styrene, toluene and xylene during working process. Mean exposure level of styrene to which workers were ex-

posed was 0.10 ppm, and for these subjects, levels of mandelic acid (MA) and phenylglyoxylic acid (PGA), which are metabolites of styrene, excreted in urine were 0.02 and 0.02 g/g creatinine, respectively. Exposure level of toluene was 0.10 ppm, and excreted hippuric acid (HA), its metabolite, in urine was 0.31 g/g creatinine; ambient level of xylene was 4.10 ppm, and methyl hippuric acid (mHA), its metabolite, was 0.09 g/g creatinine. Overall, exposure level of organic solvents to which subjects in exposed group were exposed were very low, and exposure coefficient of mixture (ECM) was evaluated as 0.05, as low as one fiftieth of ECM reference value (ECM ≤ 1) (Table 2).

Table 2.

Concentration of ambient hazardous chemicals and their metabolites in exposed workers

Variables	Mean (SD)
Styrene	
Ambient level (ppm)	0.10 (0.5)
Urinary metabolites (g/g creatinine)	
Mandelic acid (MA)	0.02 (0.02)
Phenylglyoxylic acid (PGA)	0.02 (0.01)
Toluene	
Ambient level (ppm)	0.10 (0.2)
Urinary metabolites (g/g creatinine)	
Hippuric acid (HA)	0.31 (0.27)
Xylene	
Ambient level (ppm)	4.10 (7.8)
Urinary metabolites (g/g creatinine)	
m-Hippuric acid (mHA)	0.09 (0.12)
Exposure Coefficient of Mixture	0.05 (0.01)

Threshold limit values (ppm): styrene, 20, toluene, 50, xylene, 100. Biological exposure indices (g/g creatinine): MA, 0.8, PGA, 0.24, HA, 2.5, mHA, 1.5.

Levels of catecholamine and oxidative stress index between control and exposed workers. Level of EP was 39.4 ρg/ml in control group, 43.1 ρg/ml in exposed group; level of NEP was 204.1 ρg/ml in control group, and 223.0 ρg/ml in exposed group. However, levels of these catecholamines did not show significant difference between groups. Measurement of oxidative stress status revealed that TOS was 139.1 μM H_2O_2-equivalent in control group, 139.2 μM H_2O_2-equivalent in exposed group. TAC was 317.3 μM H_2O_2-equivalent in control group and 290 μM H_2O_2-

equivalent in exposed group, and OSI (TOS/TAC) was 0.44 and 0.47 μM H_2O_2-equivalent in control group and exposed group, respectively, and TAC (p = 0.001) and OSI (p = 0.154) were not significant different between groups (Table 3).

Table 3.

Comparisons of catecholamine and oxidative stress index between control and exposed workers

Variables	Control (n = 135) mean (SD)	Exposed (n = 180) mean (SD)	p-value
EP (ρg/ml)	39.4 (24.9)	43.1 (23.3)	0.110
NEP (ρg/ml)	204.1 (119.5)	223.0 (172.3)	0.201
TOS (μM H_2O_2 equivalent)	139.1 (62.2)	139.2 (79.9)	0.990
TAC (μM H_2O_2 equivalent)	317.3 (33.8)	290 (50.7)	0.001
OSI (TOS/TAC arbitrary unit)	0.44 (0.21)	0.47 (0.27)	0.154

EN, epinephrine, NEN, norepinephrine, TOS, total oxidative stress, TAC, total antioxidative capacity, OSI, oxidative stress index.

Correlation between catecholamine, oxidative stress indices and CVD risk factors in control and exposed workers. Result from correlation analysis among CVD risk factors, catecholamines and oxidative stress indices are shown in Table 4. As expected, CVD risk factors (total cholesterol, LDL-cholesterol and HOMA-IR) showed positive correlation with metabolic syndrome criteria (triglyceride, fasting glucose) in control group and exposed group while these factors showed negative correlation with HDL-cholesterol. In control group and exposed group, EN and NEP showed association with blood pressure, and level of HDL-cholesterol. OSI showed association with lipid, i.e. total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride. For organic solvent exposure, EP showed positive correlation with ECM and styrene metabolites but only MA level showed statistically significant association (r = 0.385, p < 0.05), and NEP showed significantly positive correlation with ECM (r = 0.358, p <0 .05). Among oxidative stress indices, TOS (r = 0.401, p < 0.05) and OSI (r = 0.366, p < 0.05) showed positive correlation with ECM (Table 5). Multiple logistic regression analysis results with NCEP ATP-III metabolic syndrome criteria, which are CVD risk factors as dependent variables and EP, NEP and OSI as independent variables after adjustment to age, smoking and drinking habit, are shown in Table 6.

Table 4.

Correlation matrix adjusted to age, smoking and drinking between individual parameters related with metabolic syndrome

	SBP	DBP	T-chol	HDL-C	LDL-C	TG	FG	HOMA	EN	NEN	TO
SBP		0.867**	0.167*	−0.113	−0.041	0.288**	0.382**	0.181*	0.213**	0.104	0.
DBP	0.777**		0.150	−0.089	−0.083	0.310**	0.282**	0.126	0.158*	0.167*	0.
T-chol	0.034	0.164		0.026	0.763**	0.336**	0.103	0.029	0.015	0.016	0.
HDL-C	−0.200*	−0.155	0.078		0.071	−0.427**	−0.162*	−0.202*	0.176*	0.101	0.
LDL-C	0.035	0.099	0.869**	0.026		0.283**	0.031	0.035	−0.047	−0.034	−0.
TG	0.152	0.234	0.346**	−0.523**	−0.029		0.121	0.067	0.031	0.042	0.
FG	−0.058	0.006	0.216*	−0.005	0.138	0.233**		0.431**	−0.039	−0.127	0.
HOMA	0.051	0.039	0.190*	−0.169	0.163	0.207*	0.401**		−0.034	−0.143	0.
EN	0.108	0.061	−0.062	0.174*	−0.055	−0.135	0.045	−0.035		0.084	0.
NEN	0.175*	0.110	−0.061	−0.110	−0.074	0.100	0.026	0.198*	0.093		0.
TOS	0.042	0.056	−0.216*	−0.011	−0.089	0.270**	0.077	−0.095	0.098	0.063	
TAC	0.192*	0.278	0.057	−0.102	−0.003	0.162	0.026	0.181*	−0.059	0.083	−0.
OSI	−0.006	−0.007	−0.210*	0.006	−0.086	0.271**	−0.084	−0.130	0.119	0.043	0.

*p<0.05, **p < 0.01. SBP, systolic blood pressure, DBP, diastolic blood pressure, T-chol, total cholesterol, HDL-C, HDL-cholesterol, LDL-C, LDLcholesterol, TG, triglyceride, FG, fasting glucose, HOMA, homeostasis model assessment of insulin resistance, EN, epinephrine, NEP, norepinephrin, TOS, total oxidative stress, TAC, total antioxidant capacity, OSI, oxidative stress index. White area is control data and gray area is exposed workers data.

Table 5.

Correlation matrix adjusted to age, smoking and drinking between catecholamines and oxidative stress status with exposure level of hazardous chemicals in exposed workers

	EP	NEP	TOS	TAC	OSI
ECM	0.178	0.358*	0.401*	−0.258	−0.366*
MA	0.385*	−0.107	−0.186	0.214	−0.202
PGA	0.204	0.198	−0.201	0.149	−0.173
HA	−0.149	0.171	−0.025	0.094	−0.020
mHA	−0.196	−0.343	−0.015	−0.275	0.064

*$p < 0.05$. EP, epinephrine, NEP, norepinephrine, TOS, total oxidative stress, TAC, total antioxidative capacity, OSI, oxidative stress index, ECM, exposure coefficient of mixture, MA, mandelic acid, PGA, phenylglyoxylic acid, HA, hippuric acid, mHA, methyl hippuric acid.

Table 6.

Interrelationship between exposure coefficient of mixture and its urinary metabolites with the components of metabolic syndrome using multiple logistic regression analysis (backward)

Independent variables	Dependent variables (n = 315)					
	WC Odds (β value)	Blood pressure Odds (β value)	HDL-cholesterol Odds (β value)	Triglyceride Odds (β value)	Glucose Odds (β value)	MS Odds (β value)
Epinephrine						
Control (n = 135)	0.978 (−0.023)*	1.010 (0.010)	0.983 (−0.018)	0.980 (−0.020)	1.002 (0.002)	0.987 (−0.013)
Exposed (n = 180)	0.998 (−0.002)	1.013 (0.013)*	1.002 (0.002)	1.005 (0.005)	1.005 (0.005)	1.009 (0.009)
Norepinephrine						
Control (n = 135)	1.002 (0.002)*	1.002 (0.002)	1.001 (0.001)	1.002 (0.002)	1.003 (0.003)	1.002 (0.002)
Exposed (n = 180)	0.998 (−0.002)	1.001 (0.001)	0.996 (−0.004)	0.998 (−0.002)	0.998 (−0.002)	0.994 (−0.006)*
OSI						
Control (n = 135)	3.656 (1.297)	1.591 (0.464)	0.767 (−0.265)	0.406 (−0.990)	0.575 (−0.554)	0.947 (−0.054)
Exposed (n = 180)	0.676 (−0.392)	1.748 (0.558)	0.098 (−2.318)*	0.145 (−1.929)*	0.145 (−1.929)**	0.294 (−1.226)

*$p < 0.05$. WC, waist circumference, MS, metabolic syndrome, OSI, oxidative stress index.

In control group, waist circumference was associated with EP (Odds ratio = 0.978, $p < 0.05$) and NEP (Odds ratio = 1.002, $p < 0.05$). In exposed group, blood pressure was associated with EP (Odds ratio = 1.013, $p < 0.05$), metabolic syndrome with NEP (Odds ratio = 0.994, $p < 0.05$), and OSI showed statistically significant association with HDL-cholesterol (Odds ratio = 0.098, $p < 0.05$), triglyceride (Odds ratio = 0.145, $p < 0.05$), and glucose (Odds ratio = 0.145, $p < 0.05$).

DISCUSSION

Living organisms are continuously exposed to environmental xenobiotics, and increase the production of specific enzymes to eliminate them as a protective mechanism. Absorbed xenobiotics are excreted from the organisms through detoxification process by actions of these enzymes. Accordingly, pharmacokinetic studies and molecular level studies for internal homeostasis regula-

tors such as enzymes, neurotransmitters, cytokines, neurotrophics are being conducted to understand *in vivo* toxicity from environmental exposure to hazardous chemicals. In this study, the relation between exposure to hazardous chemicals and CVD development was investigated. In addition, laboratory study for the change in levels of homeostasis regulators in the body was performed to understand the mechanism of CVD development. CVD development is influenced by diverse factors, and in order to exclude the effect of non-modifiable risk factors, the subjects were selected taking workers' sex and age into account in this study.

The results revealed that age, drinking habit, regular excercise, and mean sleeping hours per day did not show differences between control group and exposed group, but there were more smokers in exposed group while BMI, waist and hip circumference were significantly higher in control group.

However, among NCEP-ATP III criteria known as CVD risk factors, only HDL-cholesterol showed the difference by exposure to hazardous chemicals. Increasing of HDL-cholesterol in this study may be associated with induction of cytochrome p-450. Because the cytochrome p-450 was induced by the exposure of chemicals and it was involved in the metabolism of cholesterol (Chen *et al*., 1989).

Flegal *et al*. (Flegal *et al*., 1995) reported that smoking was associated with BMI, but in this study, differences in BMI, waist and hip circumferences were not shown between smokers and non-smokers in both control group and exposed group. Energy consumption by activity is one of the factors which may affect the anthropometric results, and comparison of standing work and sitting work between control group and exposure group revealed much longer time for standing work in exposed group. It is suggested that higher BMI, waist and hip circumferences in control group than in exposed group in this study are associated with amount of energy consumption rather than the effects of smoking and exposure to hazardous chemicals. Hazardous chemicals absorbed from environment are biologically transformed by xenobiotic metabolic enzymes in the body, then are excreted from the body. During this process, metabolic intermediates and reactive oxygen species (ROS) with high reactivity and activity are produced. Metabolic intermediates are produced during metabolic process of chemicals originally absorbed but ROS such as superoxide ion

$$(O_2^-),$$

hydroxyl radical (·OH) and lipid peroxide are additionally produced during the metabolic process (Nelson, 1995). These metabolic intermediates and ROS cause cell membrane damage and disturbance and suppression upon signal transduction system, immune and endocrine system through covalent bondings with macromolecules *in vivo*, leading to homeostasis imbalance in the body and *in vivo* toxicity (Hinson *et al*., 1995), and also affect platelet aggregation and inflammatory response (Desjardins and Balligrand, 2006). Hence, this study examined the extent of oxidative stress caused by metabolic intermediates produced and ROS additionally produced during metabolic process of hazardous chemicals absorbed into the body and investigated its relation to CVD risk factors. The results showed that TOS did not change by exposure to hazardous chemicals, but OSI (TOS/TAC) increased as TAC decreased. The levels of EP and NEP, which are related with ROS

and are involved in regulating blood pressure, also increased by exposure to hazardous chemicals but did not show significant difference compared to control group. These results suggest that the levels of exposure to hazardous chemicals in exposed group were very low and did not affect directly the level of TOS and the physiological levels of EP and NEP, but reduced TAC levels by chronic exposure might increase CVD risk by ultimately decreasing the eliminating capacity for ROS and TOS. Correlation analysis for level of exposure to hazardous chemicals, catecholamines and oxidative stress showed that the level of exposure and the level of MA, a metabolite of styrene, had a positive correlation with EP, NEP, TOS and OSI. Therefore, logistic regression analysis was performed with CVD risk factors as dependent variables and EP, NEP, OSI as independent variables. The results showed that the levels of EP and NEP were associated with waist circumference in control group. However, in exposed group, EP was associated with blood pressure and NEP was associated with metabolic syndrome. In addition, OSI was not associated with CVD risk factors in control group, but was associated with HDL-cholesterol, triglyceride and blood glucose in exposed group. Catecholamines, i.e. EP and NEP are well known to be associated with the increase of blood pressure (Rosenman, 1990), and their physiological levels have been reported to be associated with ROS (Schraml *et al*., 2007). Mokuda *et al*. (1995) reported that liver damage by exposure to hazardous chemicals increased blood glucose and insulin level, and cholesterol and triglyceride were associated with the level of exposure to hazardous chemicals (Kaukiainen *et al*., 2004; Brunekreef *et al*., 2009; Allen *et al*., 2012). Heo *et al*. (2009) also reported that blood glucose increased in workers exposed to styrene. It has been reported that increased blood glucose and insulin after exposure to hazardous chemicals come from the effects of oxidative stress factors including ROS additionally produced during the metabolism of hazardous chemicals (Ericksson, 2007; Grattagliano *et al*., 2008). The observation in this study that EP, NEP and triglyceride are associated with exposure to hazardous chemicals may result from the effects of ROS and oxidative stress, and it is estimated that cytochrome P-450 isoenzymes 2B1/2 and 2E1/2 selectively induced by exposure to styrene, toluene and xylene are involved in the metabolism of cholesterol, leading to increased cholesterol and HDL-cholesterol in exposed group (Savolainen *et al*., 1990). In this study, no significant difference in anthropometric and serum chemical parameters were observed between control and exposed group. This result may be due to exposed to low levels of chemicals. Kaukiainen *et al*. (2004) reported that the feasibility of the use of liver function test remains unclear in workers exposed to low-level mixed solvents, because liver function is more affected by lifestyle than (Won *et al*., 2011) by chemical exposure.

In conclusion, overall results suggest that ROS and oxidative stress from exposure to hazardous chemicals act as mediators to change the physiological levels of EP and NEP to increase blood pressure, and ultimately increase CVD development through increase of cholesterol, triglyceride and blood glucose by lipid peroxidation.

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