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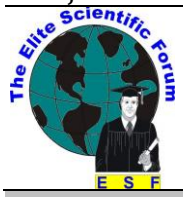

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RESEARCH ARTICLE

Occupational Exposure to Industrial Pollution Elevates the Levels of Myocardial Enzymes: A Leading Cause of Cardiovascular Abnormalities in Industrial Workers

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ABSTRACT

The effect of occupational exposure to industrial air pollution on myocardial enzymes of workers of textile, sugar and fertilizer industry and oil refinery was studied. A total of 500 male volunteers of industrial workers and non-industrial individuals with and without the history of cardiovascular disease (CVD) were included in the study. The pollutants released in working areas of selected industries and the levels of myocardial enzymes of the study groups were analyzed. The levels of particulate matter, nitrogen oxide, sulfur dioxide, and carbon monoxide were found to be higher than National Environmental Quality Standards. A significant increase (2-10 folds; $P<0.05$) was observed in the levels of creatine phosphokinase, creatine kinase-MB, lactate dehydrogenase and serum glutamate oxaloacetate transaminase of workers with and without CVD and non-industrial individuals with CVD. The observed variation in studied parameters of industrial groups without CVD may be attributed to the elevated levels of industrial air pollutants in the industrial area. However, the variation in levels of studied parameters of industrial groups with CVD may be the cumulative effect of cardiovascular abnormalities and industrial pollutants.

INTRODUCTION

The recent advancement in technology and industrialization has resulted in large-scale contamination of the global environment. Many hazardous compounds are released from industrial processing units to the working areas in the industries and nearby localities. These industrial pollutants include particulate matter (PM), Sulphur oxides (SOx), carbon monoxide (CO), heavy metals, volatile organic compounds (VOCs), ammonia (NH_3) and a group of nitrogen oxides (NOx) including nitric oxide (NO), nitrogen dioxide (NO_2), nitrogen trioxide (NO_3), nitrogen tetraoxide (N_2O_4) and dinitrogen pentoxide (N_2O_5) (Papar et al., 2012a). The combustion of baggase, the waste product of sugar industry, results in the production of PM in sugar processing, fermentation and refining units of the industry (Kushwaha 2015). In fertilizer industry, the production of mixed fertilizers has been reported to release about 300 g of NH_3, 200 g of NOx and 20 g of fluoride per ton of the fertilizer produced (Sittig, 1979). Air exhausts from spinning and dying units of textile industry contain PM and pollutant gases like CO, NOx, SOx and VOCx (Chen and Burns, 2006; Christie 2007). Oil refineries are the largest source of emission of volatile organic compounds (VOCs) in the atmosphere, which reacts with NOx in the presence of sunlight to form ground-level ozone (Mills et al., 2005; Papar et al., 2012b).

Exposure to air pollutants, particularly PM and NOx, has been found to be associated with cardiovascular diseases (CVDs) including myocardial infarction (MI), ischemic heart disease (IHD), coronary heart disease, angina pectoris and cardiovascular death (Bhatnagar 2004; Vermylen et al., 2005; Cendon et al., 2006; Simkhovich et al., 2008; Mills et al., 2009; Tonne and Wilkinson 2013; Costello et al., 2016). Diesel exhaust particles have been reported to be associated with the impairment of regulation of vascular tone, and fibrinolysis (Mills et al., 2005). Certain other ischemic

problems including atherosclerotic activation, arrhythmic symptoms, decreased heart rate variability and heart failure have also been reported to be associated with increased concentration of environmental pollutants (Holguín et al., 2003; Bhatnagar, 2004; Shah et al., 2013).

The mechanism of development of cardiovascular disease (CVD) is based on the changes and impairment of the endothelial wall which leads to atherosclerosis and finally CVD and stroke. The cardiovascular damage leads to a detectable rise in the plasma concentration of myocardial enzymes normally confined within cardiac cells (Freedman et al., 1989; Vermylen et al., 2005). The enzymes most widely used in the detection of myocardial infarction are creatin phosphokinase (CPK), creatine phosphokinase MB (CK-MB), lactate dehydrogenase (LDH) and serum glutamate oxaloacetate transaminase (SGOT) (Mythili and Malathi, 2015; Bodor, 2016).

In the light of above literature survey, it was hypothesized that the industrial air pollutants, including PM, SOx, NOx, CO, and O_3 affect the cardiac health of the industrial workers. Therefore, it seemed necessary to study the effect of environmental pollution on myocardial enzymes of individuals working in the industrial and non-industrial environments. Therefore, the present research project was planned to investigate the effect of industrial air pollutants from textile industry, sugar industry, fertilizer industry and oil refinery on the levels of myocardial enzymes level of workers.

MATERIALS AND METHODS

The study was conducted at Institute of Chemical Sciences, Bahauddin Zakariya University, Multan and Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan. The study plan was approved by the Board of Advanced Studies and Research, Bahauddin Zakariya University, Multan. A written consent was obtained from each of the participants and a questionnaire regarding the history of the participants was completed before sampling.

Analysis of industrial pollutants

The air samples were collected from the working atmosphere of textile industry, sugar industry, fertilizer industry and oil refinery and analyzed for various pollutants including particulate matter, sulfur oxides, carbon monoxide and nitrogen oxides using standard protocols described earlier (EPA) (Arnold et al., 2001; Chen and Burns, 2006; Parlar, 2006; Freeman et al., 2014).

Subjects

All the volunteers taking part in the study were subjected to clinical screening for renal, hepatic and cardiovascular diseases by a cardiac physician based on physical examination including symptomatic observations, Electrocardiograms, X-ray imaging and Echocardiography. The individuals suffering from or having a clinical history of renal and hepatic diseases and the regular smokers were excluded from the study. The non-smoker individuals without any symptoms or history of renal and hepatic diseases were included in the study and categorized further into two subgroups: 1) individuals without cardiovascular disease (CVD) and 2) individuals with CVD. The non-industrial individuals without CVD were used as a control.

The sample size was determined using statistical software G-power. The calculated sample size, at power $(1-\beta) = 0.9$ and $\alpha=0.05$, was found to be approximately 475. A total of 500 male volunteers of average age (40-50 years), weight (60-75 kg) and height (5-6 feet) were included in the study. The participants were categorized into five major groups each containing 100 individuals. The first group contained the individuals working in non-industrial localities such as offices of the university. The remaining four groups contained the individuals residing in the vicinal locality and within the boundaries of four industries including textile industry, fertilizer industry, sugar industry and oil refinery for a period of about 5-10 years.

Blood sampling

The blood sample (5 mL) was collected from each volunteer with 12 h fasting in EDTA (ethylenediamine tetra-acetic acid) vacutainer and plasma was separated by centrifugation

Analysis of myocardial enzymes

Creatine phosphokinase (CPK)

CPK activity in the sample was determined by measurement of NADPH produced in a three-step coupled enzyme reaction (Szasz et al., 1976). The creatine kinase catalyzes the dephosphorylation of creatine phosphate to creatine and conversion of ADP to ATP followed by utilization of ATP in the phosphorylation of glucose to glucose-6-Phosphate by the action of hexokinase. Finally, glucose-6-Phosphate dehydrogenase catalyzes the oxidation of glucose-6-Phosphate to 6-phosphogluconate along with the reduction of $NADP^+$ to NADPH. The concentration of NADPH is proportionate to CPK activity.

CPK standard kit 12005, Human, Germany was used to determine CPK level following manufacturer's protocols. The change in absorbance of the reaction mixture per min was noted for 3 min at 340 nm on a spectrophotometer (Micro-lab-200) and activity of CPK was calculated as:

Creatine phosphokinase activity (U/l) = ΔA/min x 1854

Where ΔA is the change in absorbance and 2854 is the calibration factor.

One unit of CPK is the amount of enzyme that will transfer 1µM of phosphate from phosphocreatine to ADP per minute at pH 6.0.

Creatine kinase MB (CK-MB)

CK-MB is an isoenzyme of CPK which consists of two subunits CK-B and CK-M. The test was performed according to the protocols as discussed for the determination of CPK without the addition of sodium azide and with complete inhibition of CK-MM activity and CK-M subunit of CK-MB using monoclonal antibodies obtained from a goat. Only CK-B activity was measured which is half of the CK-MB activity (Vaidya et al., 1986). CK-MB was determined using CK-MB standard kit: 12008, Human, Germany.

Lactate dehydrogenase (LDH)

The method is based on the reduction of pyruvate by LDH using NADH as coenzyme which is oxidized to NAD^+. The activity of LDH is determined by measuring the amount of NAD^+ produced (Decker and Lohmann-Matthes, 1988). LDH was determined by UV method using standard kit: 12014, Human, Germany. The activity of LDH was measured at 340 nm on a spectrophotometer (Micro lab-200).

Serum glutamate oxaloacetate transaminase (SGOT)

SGOT catalyzes the transamination from L-aspartate to α-ketoglutarate resulting in the formation of oxaloacetate and glutamate. The oxaloacetate thus produced is reduced to L-malate by malate dehydrogenase (MDH) in the presence of NADH. The change in NADH concentration is proportional to oxaloacetate produced by the action of SGOT (Morgenstern et al., 1966). SGOT was determined by UV method using SGOT Kit: (Cat. No. 1204), Human, Germany. The activity of GOT (U/L) was measured in terms of change in absorbance of NADH at 340 nm on a spectrophotometer (Micro-lab 200).

Statistical analysis

The sample size was determined using statistical software G-power. The results are presented as mean ± SD of the three replicates. Data were statistically analyzed by one-way analysis of variance (ANOVA) at $P \leq 0.05$. The means were differentiated by Tukey's multiple range tests using SPSS software (Version 17.0).

RESULTS

In the present study, the effect of occupational exposure to industrial pollutants on myocardial enzymes level of the workers of different industries including fertilizer industry, sugar industry, textile industry and oil refinery was studied.

Industrial pollutants

Air pollutants including PM, SOx, CO, and NOx in the working atmosphere of textile, sugar and fertilizer industry and oil refinery were analyzed by standard protocols described by Environmental Protection Agency (EPA). The observed values and National Environmental Quality Standards (NEQs) of these pollutants are given in Table 1. The concentration of PM_{Total} in the textile industry, sugar industry, fertilizer industry (CAN plant) and oil refinery was found to be 850, 1500, 513 and 1571 mg/m^3 respectively. High furnace oil boiler area of oil refinery showed the comparatively highest concentration of PM_{Total} and SO_2. The concentration of CO was found to be high in bagasse combustion exhaust area of sugar industry while NOx was found to be high at the sulfuric acid plant of the fertilizer industry. However, the levels of these pollutants were found to be higher than the NEQs for the working areas of the selected industries.

Table 1: Observed levels and National Environmental Quality Standards of different pollutants in the working atmosphere of different industries.

Industry	Sampling area	Pollutants	Pollutant conc.	NEQS*
Textile industry		PM-Total (mg/m^3)	850	300
(Sitara Chemical Industry, Faisalabad)	Exhaust area	PM-2.5 ($\mu g/m^3$)	95	15
		SPM-CL ($\mu g/m^3$)	1240	500
Sugar industry		*PM-Total (mg/m^3)	1500	300
(Shukar Gang Sugar Mill Jhang, Punjab, Pakistan)	BCE* area	SO_2 (mg/m^3)	106	1700
		CO (mg/m^3)	3350	800
		NO_x (mg/m^3)	84	400
Fertilizer industry		PM-Total (mg/m^3)	85	400
(Pak Arab Fertilizers, Multan)	Sulfuric acid plant	SO_2 (mg/m^3)	1550	500
		CO (mg/m^3)	70	800
		NO_x (mg/m^3)	140	300
	Nitric acid plant	NO_x (mg/m^3)	104	400
	CAN* plant	PM-Total (mg/m^3)	513	300
	Urea plant	Ammonia (ppm)	56	40
Oil refinery (PARCO Muzaffar Garh)		PM-Total (mg/m^3)	1571	800
	HFO* boiler area	SO_2 (mg/m^3)	5976	4250
		CO (mg/m^3)	1283	600
		NO_x (mg/m^3)	3	2

*BCE: Bagasse combustion exhaust, CAN: Calcium ammonium nitrate; HFO: High furnace oil plant; NEQs: National Environmental Quality Standards; SPM-CL: Suspended particulate matter-Cotton lint.

Table 2: The levels of myocardial enzymes (U/L) of individuals with and without CVD of non-industrial and industrial groups

Sampling area	CPK		CKMB		LDH		SGOT	
	Individuals without CVD	Individuals with CVD	Individuals without CVD	Individuals with CVD	Individuals without CVD	Individuals with CVD	Individuals without CVD	Individuals with CVD
Non-Industrial	56.86±25.43^{b*}	205.37±156.93^{b}	9.61±4.68^{b**}	28.09±12.06^{c}	109.42±8.91^{c}	254.18±57.52^{b}	16.19±6.00^{c}	53.20±27.30^{b}
Textile industry	141.87±31.98^{a}	1006.95±611.27^{a}	10.36±3.74^{b}	88.37±51.86^{b}	144.97±24.51^{a}	491.50±188.76^{a}	34.47±4.90^{a}	88.59±41.31^{a}
Sugar industry	59.59±22.11^{b}	212.22±191.93^{b}	11.50±2.46^{ab}	28.57±19.20^{c}	126.31±23.06^{b}	250.04±109.08^{b}	23.21±15.15^{b}	81.88±51.67^{a}
Fertilizer industry	138.95±32.79^{a}	1223.61±525.00^{a}	13.06±362^{a}	117.20±50.85^{a}	137.14±23.33^{ab}	535.55± 217.67^{a}	35.14±5.42^{a}	96.12±44.07^{a}
Oil Refinery	147.02±32.86^{a}	1182.91±553.95^{a}	13.46±3.24^{a}	111.20±49.70^{a}	145.07±24.04^{a}	546.93± 212.24^{a}	36.65±7.48^{a}	93.85±46.88^{a}
P value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*The results are presented as the mean±standard deviation of 50 individuals in each group. ** The means followed by the same letter, in each column, are not significantly different at P≤0.05 using Tukey's multiple range test.

Myocardial enzymes

The CPK, CK-MB, LDH and SGOT levels of individuals without CVD and those with CVD of non-industrial and industrial groups were investigated (Table 2). A statistically significant difference ($p<0.05$) from control was observed in the levels of the myocardial enzymes of individuals without CVD and those with CVD of non-industrial and industrial groups. The individuals of industrial and nonindustrial groups with CVD showed comparatively higher levels of myocardial enzymes as compared those of control group. The levels of CPK of individuals with CVD working in oil refinery were found to be higher than those of individuals working in textile, sugar and fertilizer industries. The levels of CK-MB, LDH and SGOT were also found to be comparatively high in the individuals working in the fertilizer industry and oil refinery.

DISCUSSION

The increase in environmental pollution due global industrialization is considered as an important risk factor for primary heart diseases in humans with the risk of myocardial infarction. It is generally found that the people affected by industrial air pollution usually have an atherogenic lipoprotein profile due to the effect of particulate matter. On the other hand, the increased rate of mortality from cardiovascular diseases has been found almost independent of serum lipid concentration. Therefore, in this context, the non-atherogenic mechanism seems to be more involved in the etiology of cardiovascular diseases among the people affected by industrial air pollution. The non-atherogenic mechanisms include certain factors like carboxy haemoglobin, fibrinogen and leukocytosis. It suggests that industrial pollution may cause myocardial infarction as well as the development of atherosclerosis and, therefore, is considered as an active precipitant for coronary heart disease (Utell et al., 2002; Simkhovich et al., 2008; Mills et al., 2009).

The percentage variation in pollutant concentration in working atmosphere of different industries from NEQs is represented in Fig. 1. Significant variation in the levels of air pollutants from NEQs was observed in the working areas of selected industries. PM_{Total} was found to be increased by 185, 400, 75 and 90% in the working atmosphere of textile industry, sugar industry, fertilizer industry and oil refinery respectively. SO_2 was found to be increased by 40 and 80% in the oil refinery and fertilizer industry. Sugar industry and oil refinery were also found to show 100-300% increase in CO level respectively. NOx was also found to be increased only in the oil refinery. This increase in the level of these pollutants in the working atmosphere of these industries may lead to cardiovascular abnormalities in industrial workers and the peoples residing in industrial localities.

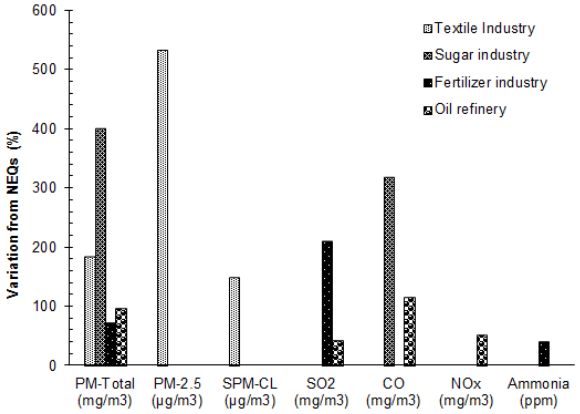

Fig. 1: Percentage variation in concentration of industrial pollutants from National Environmental Quality Standards.

The data analysis showed an elevation in the levels of CPK, CK-MB, LDH and SGOT levels of individuals without CVD of industrial groups by 9.84-171.01, 7.80-40.06, 15.44-32.58 and 43.36-126.30% respectively as compared to that of the control group. The levels of CPK, CK-MB, LDH and SGOT of individuals with CVD of nonindustrial and industrial groups were found to be increased by 262.65-2533.36, 192.30-1119.56, 117.79-397.09 and 228.60-493.70% compared to control group (Fig. 2). The levels of CPK of individuals with CVD working in the fertilizer industry and oil refinery were found to be increased 2-5 fold higher than

those of individuals working in textile and sugar industries. The levels of CK-MB, LDH and SGOT were also found to be increased more in the individuals of working in the fertilizer industry and oil refinery. The present results for the levels of CPK, CK-MB, LDH, and SGOT of control individuals and individuals of industrial groups without CVD were found to be comparable to the normal ranges reported in literature (CPK; up to 196, CKMB; up to 10, LDH; 100-200 and SGOT; 19-40 U/l) (Menzel, 1994). However, CPK, CK-MB, LDH and SGOT levels of individuals of non-industrial and industrial groups with CVD were found to be 2-10 folds higher than their normal ranges (Noakes, 1987).

The observed variation in the level of the myocardial enzyme of individuals without CVD and with CVD of industrials groups from that of the control group may be correlated with the excessive release and elevated levels of air pollutants such as PM, SOx, and CO in working atmosphere of these industries. A significant relationship was observed between the increase in the concentration of PM_{Total} and elevation in the level of myocardial enzymes in various industries particularly the textile industry and oil refinery. However, the levels of myocardial enzymes of the individuals working in sugar industry were least affected by the increase in PM_{Total}. These results are in agreement to those reported earlier that the exposure to pollutants and chemicals could elevate the risk of cardiovascular disease via mechanisms including systemic inflammation, accelerated atherosclerosis, and altered cardiac autonomic function (Bhatnagar, 2004; Pope et al., 2004).

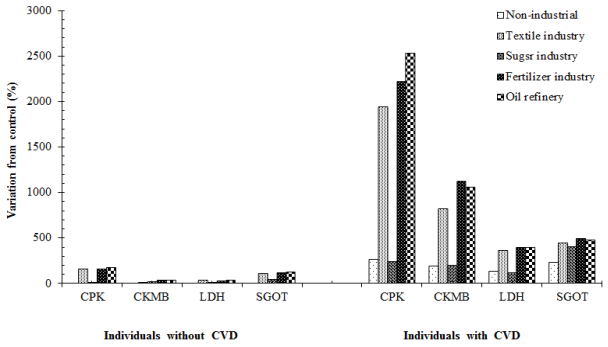

Fig. 2: Variation from control in the levels of myocardial enzymes (U/l) of individuals CVD and with and without CVD of non-industrial and industrial groups.

The relationship between air pollution, genotoxicity, and risk of CVD

Air pollution has been reported to be associated with genotoxicity in human and other experimental systems. The previous evidence has shown that air pollution causes mutation in DNA (Somers et al., 2002). A possible mechanism for increased risk of cardiovascular abnormalities due to air pollution is based on the mutation in CYP2C gene which codes for an enzyme catalyzing the production of mediators of vasodilation. Genetic variation in CYP2C8 and CYP2C9 genes results in an irregular vascular response leading to MI. The exposure to PM also causes hypermethylation of nitrogen bases in DNA which is further associated with CVD (Somers et al., 2002; Yauk et al., 2008; Muka et al., 2016).

Exposure to air pollutants, particularly PM, has been also found to generate free radicals which cause oxidative damage to DNA in human (Moller et al., 2014; Muka et al., 2016). Another proposed mechanism of pollutant generated endogenous production of ROS is based on the activation of nicotinamide adenosine dinucleotide phosphate (NADPH) oxidases. NADPH oxidase complexes produce superoxide anion by transferring an electron from NADPH to molecular oxygen which acts as a precursor of reactive oxygen and nitrogen species (Weyemi et al., 2015). The oxidative stress caused by free radicals leads to various degenerative abnormalities including atherosclerosis, chronic inflammatory disease, strokes, heart attacks and central nervous system disorders (Valko et al., 2007; Moller et al., 2014).

In the present study, the observed elevation in the levels of myocardial enzymes of industrial workers with and without CVD may be correlated with elevated levels of industrial pollutants. Based on previous evidence, it may be suggested that the exposure of elevated levels of pollutants stimulated the endogenous production of ROS which leads to the oxidative stress, DNA hypermethylation, and cardiovascular damage.

Conclusion

In conclusion, the levels of pollutants viz. PM, NOx, SOx, and CO in the working environment of various industries were found to be comparatively higher than NEQs. A significant effect of air pollution was observed on the levels of myocardial enzymes of industrial workers. Individuals of industrial groups without CVD and non-industrial individuals with CVD showed elevated levels of myocardial enzymes level. This effect was found to be multiplied by 2-10 folds in case of industrial groups with CVD. The observed variation in studied parameters of industrial groups without CVD may be attributed to the elevated levels of above-mentioned air pollutants in the working environment of the said industries. However, the variation in levels of myocardial enzymes of industrial groups with CVD may be the commutative effect of cardiovascular abnormalities and industrial pollutants. The rise in the levels of myocardial enzymes of industrial groups, as the biomarkers of CVDs, supports the hypothesis that exposure to industrial air pollutants

is a leading cause of cardiovascular abnormalities in industrial workers.

Authors' contribution

ZIZ designed the study and performed sample collection and analysis. MAS[2] developed the hypothesis and designed the study. MAS[3] supervised in experimentation and write up. HN helped in data analysis and write up.

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