

TNF- α AND IL-6 AS BIOMARKERS OF IMPAIRED LUNG FUNCTIONS IN DIMETHYLACETAMIDE EXPOSURE

TNF- α I IL-6 KAO BIOMARKERI OŠTEĆENOG FUNKCIONISANJA PLUĆA IZLAGANJEM DIMETILACETAMIDU

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Summary

Background: Tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) are well-known biomarkers of systemic inflammation that have been associated with many diseases in the past. In this study, we aimed to determine the relationship between impaired lung functions and the levels of these biomarkers in DMAc exposed people.

Methods: 101 non-exposed control subjects (Group 1) and 109 DMAc-exposed workers from the polyvinyl chloride (PVC) industry were included in the study. In the next step, the exposed group was divided into two groups according to the level of exposure (Group 2 and 3). DMAc, TNF- α , IL-6, creatinine, ALT, AST, GFR and standard spirometry measurements were carried out in all subjects.

Results: When compared to the control group, TNF- α and IL-6 levels were significantly high compatible with the increase of DMAc levels, in the exposed groups. Urinary DMAc Levels were 0.06 mg/L in the control group. This level is significantly low when compared to exposed and severely exposed group (2.43 mg/L and 3.17 mg/L). TNF- α levels were 56.86 pg/mL, 145.52 pg/mL and 230.52 pg/mL in control, exposed and severely exposed groups. IL-6 levels were found to be 38.08 pg/mL, 89.19 pg/mL

Kratak sadržaj

Uvod: Faktor tumorske nekroze- α (TNF- α) i interleukin-6 (IL-6) su dobro poznati biomarkeri sistemske upale koja je povezana s mnogim bolestima u prošlosti. U ovoj studiji nastojali smo utvrditi odnos između oštećenih funkcija pluća i nivoa tih biomarkera kod ljudi izloženih DMAc-u.

Metode: U istraživanje je uključen 101 neizložen kontrolni subjekat (grupa 1) i 109 radnika izloženih DMAc iz industrije polivinil-hlorida (PVC). U sledećem koraku izložena grupa bila je podeljena u dve grupe prema nivou izloženosti (grupe 2 i 3). DMAc, TNF- α , IL-6, kreatinin, ALT, AST, GFR i standardna spirometrijska merenja sprovedena su na svim ispitanicima.

Rezultati: U poređenju sa kontrolnom grupom, nivoi TNF- α i IL-6 bili su značajno visoki i u skladu s povećanjem nivoa DMAc kod izloženih grupa. Nivoi mokraćnog DMAc bili su 0,06 mg/L u kontrolnoj grupi. Ovaj nivo je značajno nizak u poređenju s izloženom i teško izloženom grupom (2,43 mg/L i 3,17 mg/L). Nivoi TNF- α bili su 56,86 pg/mL, 145,52 pg/mL i 230,52 pg/mL u kontrolnoj, izloženoj i ozbiljno izloženoj grupi. Utvrđeno je da su nivoi IL-6 bili 38,08 pg/mL, 89,19 pg/mL i 116 pg/mL za kontrolnu, izloženu i ozbiljno izloženu grupu. Slično, nivo FEV1/FVC

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and 116 pg/mL for control, exposed and severely exposed groups, respectively. Similarly, the FEV1/FVC ratio decreased especially in the severely exposed group ($p < 0.001$).

Conclusions: In our study, results have revealed that TNF- and IL-6 levels are promising biomarkers in the early diagnosis of lung function impairment in inhalational DMAc exposure.

Keywords: dimethylacetamide (DMAc), tumour necrosis factor- α (TNF- α), interleukin-6 (IL-6), pulmonary capacities, toxic inhalation

Introduction

The acute and chronic effects of industrial solvent exposure on the burden of occupational and environmental lung diseases are remarkable (1, 2). The harmful effects of these agents have been demonstrated in many diseases including cancer, chronic renal failure, peripheral or central neurotoxicity and developmental anomalies (3, 4).

Dimethylacetamide (DMAc) is an industrial solvent that is widely used in various applications in agrochemicals and pharmaceuticals, production of human-made fibres, industrial coatings and films (5). As it does not occur naturally, it is encountered as an anthropogenic compound. In the production of synthetic acrylic fibres, the polymerisation department where acrylic resins are prepared is the main source of DMAc exposure in the workplace. In this department, a hot water solution of DMAc is used to form acrylic fibres (wet spinning process), and it causes toxicity if protective measures are not sufficient (6). Its release to the environment, even after recovery treatment, its slow degradation and extensive use in the industry are significant public health problems (7).

Although the main route of exposure to DMAc is inhalation, dermal exposure is also possible (8). Urinary *N*-methylacetamide (NMA) is accepted as the main parameter as a biological index, but there are several studies where the direct measurement of DMAc in urine has been used (9, 10). Previous studies showed that DMAc could cause liver damage (11, 12), hallucinations (13), and delirium (14) in chronically exposed people. In an experimental study, rats were exposed to 10 to 300 ppm DMAc by inhalation for 3, 6 or 12 h/day for a total of 10 exposures. Neither testicular damage, nor irritation to upper respiratory tract were detected (15). On the other hand, there are some conflicting studies about DMAc exposure. In a prenatal *in vitro* study about inflammatory mechanisms of endotoxins, DMAc was found to regulate the expression of proinflammatory cytokines interleukin-1 β , tumour necrosis factor- α (TNF- α) and interleukin-6 (IL-6) (16).

TNF- α and IL-6 are well-known biomarkers of systemic inflammation that has been associated with many diseases in the past. These proinflammatory cytokines have also been found to be increased in

smenjen je, naročito kod izrazito izložene grupe ($p < 0,001$).

Zaključak: U našem istraživanju rezultati su pokazali da su nivoi TNF i IL-6 obećavajući biomarkeri u ranoj dijagnozi pogoršanja funkcije pluća u ekspoziciji inhalacijskog DMAc.

Ključne reči: dimetilacetamid (DMAc), faktor tumorske nekroze- α (TNF- α), interleukin-6 (IL-6), plućni kapaciteti, toksično udisanje

studies about solvent exposure to the respiratory tract (17, 18).

Despite the widespread use of DMAc in the industry, this is the first study which evaluates the association of DMAc exposure level, proinflammatory cytokines and lung function parameters. In this study, we aimed to propose a method using the relationship between cytokine levels and lung function impairment for the early clinical diagnosis of respiratory involvement in DMAc exposure.

Materials and Methods

Study Population

This case-control study was performed on non-smoker polyvinyl chloride workers examined at the Occupational and Environmental Medicine Department of the University Hospital of Bozok 2017. 210 subjects (101 control, 109 DMAc-exposed) were included in this study. The control group (Group 1) and the DMAc-exposed group consisted of local administrative staff and workers from the production unit, respectively. Inclusion criteria for the exposed group were to have high levels of urinary DMAc in their annual periodical examination, and the exclusion criteria, to have acute or chronic diseases including acute infections, chronic lung disease, diabetes mellitus, coronary vascular disease, rheumatological disease, cancer and smoking. Same exclusion criteria were applied to the control group. In the further analysis, the case group was divided into 2 groups according to the levels of exposure: exposed group (Group 2) (urinary DMAc=1–3 mg/L) ($N=71$, urinary DMAc levels between 1.12–2.75 mg/L) and severely exposed group (Group 3) (urinary DMAc ≥ 3 mg/L) ($N=38$, urinary DMAc levels between 3–3.75 mg/L). Informed consent forms were taken from all participants. The ethical consent was approved by Keçiören Research and Educational Hospital Ethical Committee.

Collection of Biological Samples and Biochemical Analysis

Blood samples were taken from each participant to evaluate immunologic parameters. The sera of the

blood samples were separated by centrifugation at 1500 rpm for 10 minutes. Serum samples were transferred to 2 mL Eppendorf tubes and stored at -20°C . Then, samples were transferred to the Occupational and Environmental Toxicology Laboratory of Yozgat Bozok University Science and Technology Application and Research Center (B LTEM) in a cold chain environment. Samples were stored at -20°C until analysis. IL-6 and TNF- α levels were then studied by dissolving the samples. ELISA Kits (DIA-source, catalogue numbers: KAP1261 and KAP1751, respectively) were used for IL-6 and TNF- α analyses. After the necessary pre-treatments had been made, samples were placed on microplates. Afterwards, microplates were carried to an ELISA (BMG LAB-TECH ClarioStar model) for reading. The wavelength was set at 450 nm. 5-point calibration curves were used for IL-6, and TNF- α measurements. The r^2 values of the calibration curves were calculated as 0.9994 and 0.9992, respectively. The control sample which was ready in the kit was used for verification. Linear regression was performed to obtain IL-6 and TNF- α results.

Urine samples were collected from each participant and placed in glass tubes with screw caps, and immediately placed on ice and stored at -20°C . The DMAc levels of urine samples measured with Gas Chromatography-Mass Spectrometry (GC-MS) system (Shimadzu QP2010 ULTRA). 0.2 mL of urine was treated with 0.8 mL of 2-propanol. This mixture was shaken and then dried with 0.2 g of sodium sulfate Na_2SO_4 (Sigma Aldrich). Then, the final mixture was centrifuged for 10 minutes at 3000 rpm. A 0.2 mL of an aliquot of supernatant was again mixed with 0.8 mL of 2-propanol, then 1 μL was drawn for GC-MS analysis.

An autosampler injection system coupled with a Shimadzu-QP2010 ultra (Kyoto, Japan) gas chromatography plus a mass spectrometric detector was used for DMAc analysis as described by Perbellini et al. (9). The injection volume was 1 μL . The calibration curve was constructed using six different concentrations (0.05 mg/L, 0.1 mg/L, 0.5 mg/L, 1 mg/L, 2.5 mg/L and 5 mg/L) of dimethylacetamide (Sigma Aldrich).

Spirometry Measurements

Pulmonary function tests were applied to all participants. Standard spirometry measurement was carried out by a dry-seal-spirometry (Zan 100, Spire Health Inc., Oberthulba, Germany). Lung function tests were defined in accordance with the American Thoracic Society standards (19). Lung function test data included forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), FEV1/FVC actual, and peak expiratory flow (PEF).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS 24, Inc., Chicago, IL, USA) was used for statistical analysis. The t-test was carried out to determine participants' characteristics among the control and case groups (Table I). Pearson test was used to evaluate the correlations between lung function parameters and toxicological-biochemical variables among the two groups (Table II). One way ANOVA was used to compare the averages of more than 2 independent variables among the three groups. All tests were considered significant at $p < 0.05$.

Results

The total number of participants were 210 males including control group with 101 subjects and DMAc-exposed group with 109 subjects. There was no statistically significant difference between groups for ages (34.4 ± 6.4 years for control and 35.8 ± 6.7 years for main study groups, $p=0.121$). Urinary DMAc levels, TNF- α and IL-6 were significantly higher in the DMAc-exposed group than the control group ($p < 0.001$) while the values of FEV1 and FVC were significantly lower in the DMAc-exposed group than control group ($p < 0.001$) (Table I). No difference between groups was observed for FEV/FVC, PEF, creatinine, ALT, and AST (Table I).

In Pearson correlation analysis, strong positive correlations between urinary DMAc levels and serum TNF- α ($r = 0.895$, $p < 0.001$), serum IL-6 ($r = 0.869$, $p < 0.001$) and a negative correlation with FVC ($r = -0.873$, $p < 0.001$), FEV1 ($r = -0.867$, $p < 0.001$) and FEV1/FVC ($r = -0.150$, $p < 0.001$) were found (Table II). There was no correlation between urinary DMAc levels and serum ALT, AST, GGT, GFR, creatinine and age ($p > 0.05$ for all).

In further analysis, the DMAc-exposed group was divided into 2 groups, the exposed one (Group 2) ($n=71$) and the severely exposed one (Group 3) ($n=38$) as mentioned in the method section. The levels of DMA, TNF- α and IL-6 were significantly different and had an increasing pattern from control (Group 1) to the severely exposed group ($p < 0.001$) (Table III). FEV1 and FVC values in litres had a decreasing pattern from Group 1 to Group 3, and there was a significant difference between groups. (FEV1: 4.52, 3.68, 3.03; FVC: 5.04, 4.10, 3.53, respectively) ($p < 0.001$). The ratio of FEV1/FVC was the lowest in the severely exposed group when compared to the others (0.89, 0.89, 0.86, respectively) ($p < 0.001$) (Table II). The difference between PEF, hepatic enzymes and renal markers was not significant between groups ($p > 0.05$).

Table I Main Laboratory Parameters of Group 1 (Control) and Group 2 + Group 3 (DMAc-exposed).

	Control (n=101) (Group 1)	DMAc-exposed (n=109) (Group 2 + Group 3)	P
Dimethylacetamide (mg/L)**	0.05±0.04	2.68±0.42	<0.001*
TNF- α (pg/mL)	56±30	175±49	<0.001*
IL-6 (pg/mL)	38±21	98±19	<0.001*
FEV1 (L)	4.52±0.39	3.45±0.39	<0.001*
FVC (L)	5.04±0.42	3.90±0.38	<0.001*
FEV/FVC	0.89±0.06	0.88±0.05	0.079
PEF (L/min)	8.33±1.56	8.23±1.56	0.651
Creatinine (μ mol/L)	64.53±14.14	66.30±15.02	0.449
ALT (U/L)	23±12	26±16	0.129
AST (U/L)	24±8	25±9	0.201
GFR (mL/min)	138±50	132±46	0.401

TNF- α : Tumour necrosis factor; IL-6: Interleukine 6; FEV: forced expiratory volume; FVC: forced vital capacity; PEF: peak expiratory flow; AST: Aspartate transaminase; ALT: Alanine transaminase; GFR: Glomerular filtration rate. Values were presented as mean±standard deviation. *p<0.001: significant, **urinary level

Table II Pearson Correlation (r) Coefficients of Group 1 and Group 2–3.

	DMA	TNF	IL6	FEV1	FVC	FEV1 /FVC	PEF
DMA	1						
TNF	0.895**	1					
IL6	0.869**	0.940**	1				
FEV1	-0.867**	-0.901**	-0.876**	1			
FVC	-0.873**	-0.860**	-0.835**	0.913**	1		
FEV1 /FVC	-0.150*	-0.262**	-0.245**	0.377**	-0.027	1	
PEF	-0.028	0.000	-0.008	0.039	-0.007	0.116	1

** p< 0.01; * p< 0.05; N=210

Table III Distribution of DMA, TNF- α , IL-6, pulmonary function tests, ages and routine biochemical tests among the 3 groups.

	Group	Mean	Std. Deviation	P
Dimethylacetamide (mg/L)**	1	.06	.05	<0.001*
	2	2.43	.25	
	3	3.17	.22	
TNF- α (pg/mL)	1	56.86	30.06	<0.001*
	2	145.52	23.17	
	3	230.52	36.61	
IL-6 (pg/mL)	1	38.08	21.05	<0.001*
	2	89.19	10.28	
	3	116.16	21.70	
FEV1 (L)	1	4.52	.39	<0.001*
	2	3.68	.19	
	3	3.03	.33	
FVC (L)	1	5.04	.43	<0.001*
	2	4.10	.22	
	3	3.53	.35	
FEV1/FVC (L)	1	.89	.06	<0.001*
	2	.89	.05	
	3	.86	.04	
PEF (L/min)	1	8.33	1.56	0.6
	2	8.15	1.61	
	3	8.39	1.46	
Creatinine (μ mol/L)	1	64.53	14.14	0.6
	2	67.18	15.91	
	3	65.41	15.90	
ALT (U/L)	1	23.45	12.60	0.28
	2	27.01	16.46	
	3	25.57	15.63	
AST (U/L)	1	24.09	8.02	0.17
	2	26.45	8.98	
	3	24.08	9.60	
AGE	1	34.40	6.48	0.085
	2	36.50	6.40	
	3	34.30	6.70	

TNF- α : Tumour necrosis factor; IL-6: Interleukine 6; FEV: forced expiratory volume; FVC: forced vital capacity; PEF: peak expiratory flow; AST: Aspartate transaminase; ALT: Alanine transaminase; GFR: Glomerular filtration rate. Values were presented as mean \pm standard deviation. *p<0.001: significant, **urinary level

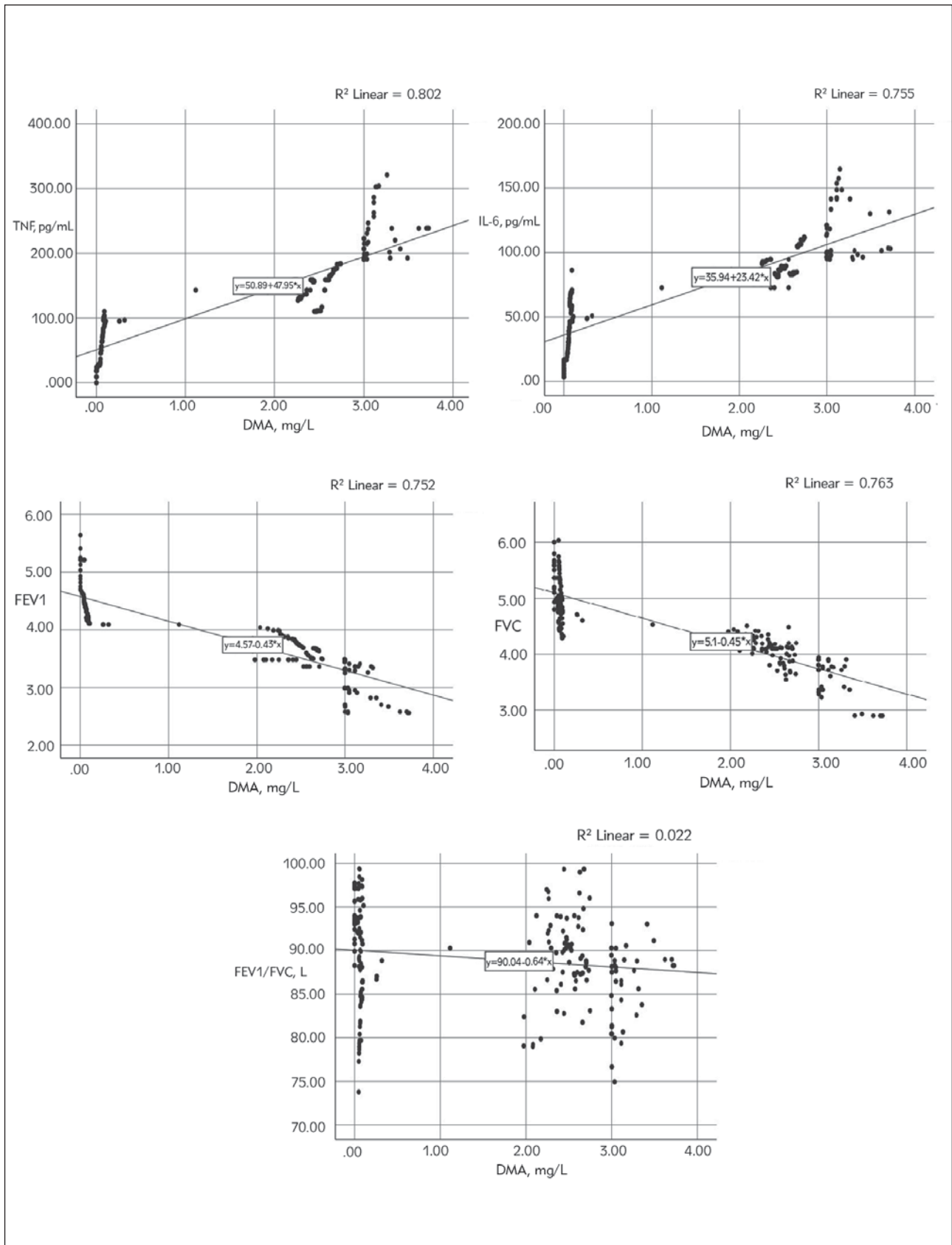


Figure 1 R² values and scatter graphics DMAc and TNF- α , FVC, IL-6, FEV1, FEV1/FVC.

Discussion

To our knowledge, this is the first study that evaluates the association between proinflammatory cytokines and impaired lung functions in DMAc-exposed people. The similarity between ages and characteristics of the groups gave us the opportunity to have similar cutoff levels for biochemical values. In DMAc-exposed group DMAc, TNF- α and IL-6 were detected significantly higher than the control group ($p < 0.001$). Additionally, DMAc had a positive correlation with serum TNF- α ($r = 0.895$, $p < 0.001$), and serum IL-6 ($r = 0.869$, $p < 0.001$) levels. Although there are studies that support the correlation between DMAc exposure and inflammation, it is possible to see some conflicting results in a few publications. Sundaram et al. (16) studied the proinflammatory response in endotoxin-induced preterm birth in mice and suggested that DMAc mediated the regulation of the expression of cytokines (IL- β , TNF- α and IL-6), supporting the evidence of the anti-inflammatory role of DMAc. In another study, Chinese researchers conducted a cross-sectional study among trichloroethylene-exposed workers where no significant differences in levels of IL-6 or TNF- α were observed (20). However, the increased levels of TNF- α and IL-6 in our study seem to demonstrate the proinflammatory response to DMAc exposure.

In our study, FEV1 and FVC values were found significantly lower in the DMAc-exposed group than the control group ($p < 0.001$). The difference between the groups was not observed for FEV/FVC, PEF, creatinine, ALT and AST values. DMAc levels were negatively correlated with FVC ($r = -0.873$, $p < 0.001$). Similar findings have been presented in an environmental study that investigates the relationship between petrochemical pollution and lung function testing parameter (21). In another case-control study on respiratory findings of workers who were exposed to industrial solvents in a gun factory, the same changes on the lung capacities were observed (22). The FEV1/FVC levels were found to be lowest in the severely exposed group when

compared to the others ($p < 0.001$) in our study. This indicates the decrement in pulmonary capacities as the exposure intensity increases. Irritation to lungs may be expected by DMAc exposure via inhalation, but the literature was limited to a few studies. 40 ppm of DMAc exposure caused minimal signs of irritation on the lungs of rats histopathologically, while 100 ppm and above levels caused clinically significant dose-related upper respiratory tract toxicity (23). Although previous occupational studies in acute and chronic exposure to DMAc confirmed liver toxicity relying on ALT and AST level alterations (8, 11, 12), our results are not consistent with these findings.

Limitations of this study were lack of DMA air measurements of the workplace environment and selection bias due to the design of the study. Strengths of this study were being the first study evaluating DMA exposure and pulmonary function test associations, and the elimination of confounding factors such as smoking and having chronic diseases.

Conclusion

Organic solvent exposure is an important health hazard that can occur occupationally and environmentally. DMAc exposure may trigger proinflammatory responses and cause a decrement in lung functions among polyvinyl chloride workers. In the future, in order to identify the underlying patterns of DMA exposure levels in workers, biomonitoring could be improved, and pulmonary function tests could be carried out on these workers with pro-inflammatory marker levels. Future studies in a cohort setting should be designed to enlighten the causation between DMA exposure and pulmonary function disturbances.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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