

SERUM CLARA CELL PROTEIN (CC16) IN RELATION TO PULMONARY FUNCTIONS AMONG WORKERS EXPOSED TO NAPHTHALENE IN PVC MANUFACTURE

By

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Abstract

Introduction: Naphthalene is a harmful environmental tox reduced in serum and/ or airway lining fluid of patients with COPD, asthma, bronchopulmonary dysplasia, silicosis, and post-transplant rejection. **Aim of work:** This study was done to evaluate the use of serum CC16 level as a biological marker for occupational respiratory diseases in relation to pulmonary function tests among workers exposed to naphthalene in polyvinyl chloride (PVC) manufacture. **Material and methods:** The study included 33 exposed workers and 33 control subjects. The study groups were subjected to full history taking, clinical examination and pulmonary function testing. Serum levels of CC16 and naphthalene levels measured for both groups. **Results:** Pulmonary functions parameters (FVC, FEV1, FEV1/FVC, FEF 25%, FEF 50%, FEF 75%), were significantly decreased in the exposed group compared to the control and indicated obstructive ventilatory impairment among the exposed workers. Serum naphthalene was significantly higher, while serum CC16 was significantly lower among the exposed group compared to the control. Exposed smokers exhibited the highest serum naphthalene and the lowest serum CC16 among all study groups. The duration of naphthalene exposure positively correlated with the serum level of naphthalene and negatively correlated with pulmonary functions and the serum level of CC16. Also serum level of CC16 correlated negatively with the serum naphthalene level and smoking index. **Conclusion:** Occupational exposure to naphthalene in PVC manufacture is associated with obstructive ventilatory impairment, with corresponding decrease in the serum

level of Clara cell protein (CC16), which can be used as a biomarker for respiratory system affection among workers exposed to naphthalene.

Key words: Naphthalene, Clara cell protein (CC16), Polyvinyl chloride (PVC), Interferon- γ , Tumour necrosis factor- α and Pulmonary functions tests.

Introduction

Naphthalene is a white solid substance that evaporates easily. It is present in petroleum and coal, and has been traditionally used in mothballs and moth flakes. Cigarette smoke contains naphthalene and it is also produced during burning wood. It has a strong but not bad smell. The most common industrial use of naphthalene is in the manufacture of polyvinyl chloride (PVC) plastics (De Pooter, 2013).

Naphthalene is a harmful environmental toxin, which selectively injures non-ciliated Clara cells present in the terminal bronchioles (Yildirim et al., 2008). Previous studies have suggested that low-molecular weight proteins (LMWP) specific for the lung might serve as peripheral biomarkers of lung toxicity. A lung biomarker known as Clara cell (CC16) can be measured in the serum, bronchoalveolar fluid (BAL) and sputum has recently been identified as the major secretory product of the lung (Gil and Pla, 2001). This protein occurs in high concentrations in the epithelial lining fluid where it plays an important antioxidant and anti-inflammatory role

by the production and/or activation of phospholipase-A2, interferon- γ and tumour necrosis factor- α (Broekaert and Bernard, 2000).

Clara cell protein (CC16) is secreted all along the tracheobronchial tree and especially in the terminal bronchioles where Clara cells are localized. It has an important role in the protection of respiratory tract against oxidative stress and inflammatory response. However, it is of major interest as a peripheral lung biomarker for assessing the cellular integrity or the permeability of the lung epithelium (Broekaert et al., 2000). The normal range of serum CC16 is 3.7- 24 ng/ml (Shijubo et al., 2003).

Changes of serum CC16 concentration may result from three mechanisms: increased intravascular leakage of CC16 from the lung across the damaged lung epithelium barrier; decreased production of CC16 in the respiratory tract and hence decreased efflux of the protein into serum; or reduction of the clearance of CC16 from plasma due to renal insufficiency

(Broekaert et al., 2000).

Several studies have shown that levels of Clara cell secretory protein are dramatically reduced in serum and/or airway lining fluid of patients with Chronic obstructive pulmonary disease (COPD), asthma, broncho-pulmonary dysplasia, silicosis, and post-transplant rejection (Wattiez et al., 2000; Hermans et al., 2001; Pilette et al., 2001 and Mattsson et al., 2005).

Joshua et al., 2010, did an experiment on 2 types of mice: one exposed to naphthalene (which will lead to Clara cell ablation and cause CC16-deficiency) the other is a control wild mice. Both groups were exposed to inhaled oxidant pollutants. The first group showed increased lung inflammation in comparison to the control group.

Aim of work

This study was done to evaluate the use of serum Clara cell protein (CC16) level as a biological marker for occupational respiratory diseases in relation to pulmonary function tests among workers exposed to naphthalene in polyvinyl chloride (PVC) manufacture.

Materials and methods

- **Study design:** This study is an observational comparative cross-sectional cohort study.
- **Place and duration of the study:** This study was done in a factory of polyvinylchloride (PVC) in Obour city, Cairo-Ismailia desert road. It was done during the period of 6 months from January to July 2018.
- **Study sample:** The study included an exposed and a non-exposed group. The exposed group included all the workers in the plant who met the inclusion criteria. They were 33 male workers. The non-exposed group included 33 patients who visited the fitness and rehabilitation unit for pulmonary function testing as a pre-operative assessment. They have never worked in PVC manufacture and had no previous exposure to airborne dust, particles or noxious gases at their workplace.

Inclusion criteria: Workers included in the study should have been working in the manufacture of PVC for at least 6 months whether in this plant or in another one. Control group included pre-operative patients with matching age and smoking habits, with no history of occupational or environmental exposure to naphthalene. Exclusion

criteria: Workers with any other exposure in another job outside this plant were excluded. Control patients with history suggestive of exposure to naphthalene or working in PVC or plastic industry were excluded.

- **Study methods:** The study groups were subjected to the following:

1- Pre-designed questionnaire including full medical and occupational history taking.

2- Clinical examination including general examination and local chest examination

3- Pulmonary function tests: Forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), FEV1/FVC, peak expiratory flow rate (PEF), forced expiratory flow rates (FEF25%, FEF50% and FEF75%) were measured for the studied groups using a portable spirometry (Ganshorn Medizin Electronic).

4- Laboratory investigations:

- **Serum CC16 level:** A blood sample of 3 ml was drawn through venipuncture of the arm using sterile plastic syringe. Blood was allowed to clot by leaving it undisturbed at room temperature for 10-20 minutes. The

clot was removed by centrifugation for 20 minutes at 2000-3000 rpm. The samples were stored at -20°C until ELISA assay was performed for all samples. Serum level of Clara cell protein (CC16) was measured in ng/ml using 'Human Clara Cell Protein, CC16 ELISA Kit' provided by BioVendor research and diagnostic products (www.biovendor.com). This ELISA kit uses Sandwich-ELISA as the method. It can be used to assay CC16 levels in human serum, plasma, culture media or any biological fluid.

- **Serum Naphthalene level** was measured for the study group using high performance liquid chromatography (HPLC) with ultraviolet (UV) detector (Kamal et al., 2011).

Consent

An informed verbal consent was taken from subjects who agreed to participate in the study before the start of work with assurance of confidentiality and anonymity of data.

Ethical approval

Approval from the administrative authority of the factory was obtained. The study protocol was approved by the Ethical Committee of the department of Occupational and Environmental

Medicine, Faculty of Medicine, Cairo University (Research Ethics Committee: approval N-158-2018).

Data management

Data were analyzed using Statistical Package for Social Science version 17 (SPSS 17). The mean values and standard deviation (SD) were estimated for the quantitative variables, while the qualitative variables were presented as numbers and percentages. Comparisons between the exposed and control groups were done using Chi square test for qualitative variables and independent

samples t- test for normally distributed quantitative variables. Meanwhile, the nonparametric Mann-Whitney test was used for not normally distributed quantitative variables. One-Way ANOVA test and post-hoc test (Turkey multi-comparison test) were used to compare between more than two groups with nominal data. Correlations were done to detect the linear relations between quantitative variables. p values less than 0.05 ($p < 0.05$) were considered statistically significant, and p values less than 0.001 ($P < 0.001$) were considered highly statistically significant.

Results

Table (1): Demographic characteristics of the exposed and control groups.

Items	Exposed group (No =33) Mean \pm SD		Control group (No =33) Mean \pm SD		t /Z#	p value
Age/ years	44.23+ 9.65		42.32+8.43		0.73	>0.05
Weight/ Kg	87.06+6.03		85.34+5.74		0.67	>0.05
Height/ m	1.75+3.54		1.78+4.2.1		1.81	>0.05
Smoking index##	16.28+6.65		18.72+5.17		1.67#	>0.05
Smoking habit	No	%	No	%	X ²	>0.05
	Smokers					
Non-smokers	11	33.3%	9	27.2%	0.72	>0.05
	22	66.6%	24	72.7%		

Z#: Mann Whitney for non-parametric data (Smoking index)

##: Smoking index= Number of cigarettes/day \times Number of years.

Table (1) showed that there was no statistically significant difference ($p>0.05$) between the exposed and the control groups as regards age, weight, height, and smoking habit.

Table (2): Comparison between the exposed and the control groups as regards some pulmonary function tests.

Parameters	Exposed group No =33 Mean \pm SD	Control group No =33 Mean \pm SD	Z	p value
FVC%	81.13+ 7.32	89.91+ 3.17	3.01	<0.05*
FEV1%	73.32+ 9.52	83.65 +16.54	4.12	<0.001**
FEV1/FVC	75.83+ 7.43	85.83+ 13.73	3.74	<0.001**
PEF%	65.63+ 15.13	83.07+ 5.30	7.41	<0.001**
FEF25%	71.64 +14.76	91.67 +15.54	4.23	<0.001**
FEF50%	73.46+ 9.44	83.20 +6.81	3.63	<0.001**
FEF75%	64.52+ 18.91	81.34 +9.78	5.53	<0.001**

*Statistically significant

**Highly statistically significant

Table (2) showed that the forced vital capacity (FVC%) was within normal range in both groups but significantly ($p<0.05$) higher among the control group. Moderate obstructive impairment was detected among the exposed group (FEV1<80% of predicted) which was statistically highly significant when compared to the control group. Also, there was highly statistically significant decreased peak expiratory flow rate (PEF%), forced expiratory flow rate 25% (FEF25%), forced expiratory flow 50% (FEF50%) and forced expiratory flow 75% (FEF75%) when compared to the control group.

Table (3): The serum levels of Naphthalene and Clara cell protein (CC16) in the studied groups.

Items	Exposed group No =33	Control group No =33	Z	p value
Naphthalene level (ng/ml)	27.42+5.31	5.31 +2.75	6.63	<0.001**
CC16 (ng/ml)	1.43 + 0.96	12.66 + 6.02	6.56	<0.001**

**Highly statistically significant

Table (3) showed that there was a highly statistically significant difference ($p < 0.001$) between the exposed and the control groups as regards serum levels of naphthalene and CC16.

Table (4): Comparison between exposed and controls (smokers and non-smokers), regarding serum Naphthalene level and CC16.

Variables	Exposed smokers No=11	Exposed non smokers No=22	Control smokers No =9	Control non smokers No =24	One-Way ANOVA test	
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	F	p value
Serum Naphthalene (ng/ml)	36.03+ 5.05	23.13+ 9.76	9.33+ 3.27	4.08+ 1.24	82.11	<0.001**
CC16 pg/ml)	0.46 + 0.19	1.91+ 0.80	3.77 +1.78	16.21 + 2.63	18.36	<0.001**

**Highly statistically significant

Table (4) showed a statistically significant increase in the serum level of naphthalene and a statistically significant decrease in the serum level of CC16 among exposed smokers compared to other groups using one-way ANOVA test and post-hoc test (Turkey multi-comparison test) .

Table (5): Correlation between duration of exposure and serum level of CC16 with other measurable parameters.

Parameters	Duration of Naphthalene exposure		CC16	
	r	p value	r	p value
Serum Naphthalene	0.46	<0.05*	-0.73	<0.001**
CC16	-0.75	<0.05*	–	–
FVC %	-0.63	<0.05*	0.86	<0.001**
FEV1 %	-0.48	<0.001**	0.37	<0.05*
FEV1/FVC %	-0.35	<0.001**	0.29	<0.05*
PEF %	-0.31	<0.001**	0.41	<0.05*
FEF25 %	-0.54	<0.001**	0.76	<0.05*
FEF50 %	-0.65	<0.001**	0.53	<0.05*
FEF75 %	-0.38	<0.001**	0.51	<0.001**
Smoking index	–	–	-0.69	<0.05*

*Statistically significant

**Highly statistically significant

Table (5) showed that there was a statistically significant negative correlation between all pulmonary function parameters and the duration of naphthalene exposure. Meanwhile, there was a statistically significant positive correlation between pulmonary functions and the serum level of CC16. Also, there was a statistically significant negative correlation between the serum level of CC16 with the serum naphthalene level and smoking index. The duration of naphthalene exposure positively correlated with the serum level of naphthalene and negatively correlated with the serum level of CC16.

Discussion

Airway epithelial cells might be exposed to environmental toxicants that result in airway injury. Exposure to naphthalene is thought to cause site-selective damage to Clara cells in the terminal airways (Yildirim et al., 2008).

The objective of our study was to evaluate the use of serum Clara cell protein (CC16) level as a biological marker for occupational respiratory diseases in relation to pulmonary function tests among workers exposed to naphthalene during the manufacture of PVC.

The study included 33 exposed workers and 33 control subjects. Demographic characteristics of the study groups showed that no statistically significant difference was found between exposed and non-exposed groups as regards age, weight, height, and smoking habit (Table 1).

Pulmonary functions parameters (FVC, FEV1/FVC, PEFR, FEF25%, FEF50% and FEF75%) were significantly higher among the control group compared to the exposed group (Table 2). However, mean FVC was within the normal range (>80% of predicted) in both exposed and non-exposed groups, while mean FEV1/FVC was below normal (<70%) indicating obstructive impairment among the exposed workers.

This was consistent with the results obtained by Yildirim et al. (2008) who studied keratinocyte growth factor as a protector against Clara cell injury induced by naphthalene. They found that acute airway epithelial damage caused by naphthalene was associated with significant airway dysfunction in mice as measured by head-out body plethysmography.

Also our work was in agreement with the results obtained by Abdel-Salam et al. (2014). They studied the relation between serum level of naphthalene and 1,2 benz-anthracene, and the immunologic markers of asthma among children in Egypt. They found that spirometric parameters (FVC, FEV1, FEV1/FVC) were significantly lower in asthmatic children which were proved to be environmentally exposed to polycyclic aromatic hydrocarbons (PAHs) as their serum level of naphthalene and 1,2 benz-anthracene were significantly higher than the control group.

The current study showed that serum naphthalene was significantly higher among the exposed group compared to the control (Table 3). This was in accordance with the results observed by Sepai and Sabbioni (2017) who studied albumin adducts and urinary metabolites resulting from occupational exposure to 1,5-naphthalene diisocyanate among workers of plastic industry. They reported that 1,5-Naphthalenediamine was found in about 60% of the studied samples which were obtained after acid hydrolysis of plasma, albumin, and

urine of exposed workers.

Serum Clara cell protein was significantly lower among the exposed compared to the control group (Table 3). This was consistent with the results detected by Joshua et al. (2010). They investigated the role of Clara cells in attenuating the inflammatory response through regulation of macrophage behaviour in mice. They stated that Clara cells were specifically ablated by naphthalene exposure which will lead to diminution of their secretory functions.

Also our work was in agreement with the results obtained by Yildirim et al. (2008) who conducted an experimental study on mice. They found that after injection of naphthalene, the number of Clara cells per square millimetre of basement membrane of the distal airways was reduced to 50%. Also CC16 was significantly reduced in the naphthalene-treated mice.

Comparison between smokers and non-smokers among exposed and control groups, revealed that exposed smokers had the highest serum naphthalene level, followed by the exposed non-smokers, then the control smokers and finally the control non-smokers who

had the lowest naphthalene level (Table 4). This difference was statistically significant ($p < 0.001$). This could be explained by the fact that cigarette contains naphthalene (De Pooter, 2013), which adds to the occupational exposure among exposed smokers, and lead to higher levels of serum naphthalene among control smokers than control non-smokers.

This was consistent with the results obtained by Kamal et al. (2011) who studied the biological monitoring of blood naphthalene level as a marker of occupational exposure to polycyclic aromatic hydrocarbons (PAHs) among auto-mechanics and spray painters in Rawalpindi. They found that exposed smokers had significantly higher levels of naphthalene in their blood than exposed non-smokers.

The results of our study agreed with that detected by Wasserman et al. (2018) in their study done to compare biomarkers of tobacco exposure between premium (expensive) and discount brand (low price) cigarette smokers. They concluded that smokers exhibited high serum concentrations of polycyclic aromatic hydrocarbons,

including naphthalene, and that discount brand cigarette smokers had higher serum levels of these compounds.

The current study showed that exposed smokers had the lowest serum CC16 compared to other groups, followed by the exposed non-smokers, then the control smokers and finally the control non-smokers (Table 4). This was similar to the findings of the study done by Lomas et al. (2009) on steroid sensitive serum surfactant protein D associated with exacerbations of COPD. They stated that significantly reduced serum CC16 levels were detected in healthy smokers and COPD patients compared with non-smokers controls.

It was also in accordance with the results declared by Lacho-Contreras et al. (2015) who reported that exposing mice to cigarette smoke reduced airway CC16 expression.

Serum level of naphthalene correlated positively with the duration of exposure among the exposed workers (Table 5). This was in agreement with the results obtained by Kamal et al. (2011) who concluded that blood naphthalene concentration showed a strong correlation with work hours and

years spent in job.

Serum level of CC16 among the exposed workers correlated negatively with serum level of naphthalene and duration of exposure (Table 5). This was on contrary to the results obtained by Bergamaschi et al. (2003), who studied indicators of pulmonary epithelial damage among foundry workers exposed to airborne pollutants including naphthalene as one of the PAHs. They reported that urinary naphthol (naphthalene metabolite) did not correlate with the serum CC16 among the exposed workers. This disagreement might be due to the difference in the sample type used for biomonitoring of naphthalene in the two studies (serum or urine). Further studies are needed to clarify this relation.

Serum level of CC16 among the exposed group correlated positively with the pulmonary functions parameters of the same group (Table 5). This agreed with the results obtained by Lacho-Contreras et al. (2015). They studied the protective role for CC16 in the development of COPD and found that CC16 expression was reduced in the large airways of chronic obstructive

pulmonary disease (COPD) patients and that expression levels decreased with increasing airflow limitation. They concluded that airway CC16 expression is reduced in human smokers and COPD patients, and correlates indirectly with COPD severity.

This also agreed with the results obtained by Laucho-Contreras et al. (2016). They suggested that low serum CC16 could serve as a useful predictive biomarker of rapid decline in FEV1 in COPD patients and that low plasma CC16 is associated with chronic bronchitis and decline in lung functions even before subjects develop COPD.

Moreover, serum CC16 correlated negatively with the smoking index (Table 5). This was in agreement with the results declared by Bernard et al. (1993), who studied serum Clara cell protein as an indicator of bronchial cell dysfunction caused by tobacco smoking. They found that the concentration of CC16 in serum was decreased in a dose-dependent way by tobacco smoking, and also reported that the concentration of the serum CC16 was decreasing on average by 15% for each 10-pack-year.

A significant negative correlation

was found between pulmonary function parameters and duration of exposure to naphthalene among the exposed group (Table 5). This was consistent with the results obtained by Cakmak et al (2014), who studied residential exposure to volatile organic compounds (VOCs), including naphthalene, and lung functions in a population-based cross-sectional survey. They reported that increase in measured indoor VOCs concentrations, including naphthalene, was associated with significant decrease in pulmonary function parameters among the study population, especially FEV1 and FEV1/FVC ratio.

Conclusion

Occupational exposure to naphthalene in PVC manufacture was associated with obstructive ventilatory impairment, with corresponding decrease in the serum level of Clara cell protein (CC16). The latter can be used, as a biomarkers for respiratory system affection among workers exposed to naphthalene.

Conflict of interest

Authors declared that no conflict of interest exists.

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