

CLARA CELL PROTEIN CC16 AS A BIOLOGICAL MARKER OF LUNG INJURY AMONG QUARRY WORKERS EXPOSED TO PM2.5

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ABSTRACT

Objective: evaluation of the use of Clara cell protein (CC16) level in serum as an early indicator of the adverse respiratory health effects of particulate matter (PM2.5) exposure.

Methods: Detailed assessment questionnaire was used for interviewing 54 individuals divided into workers exposed to particulates in the atmosphere of stone quarries and a control group. The studied population was subjected to clinical examination and pulmonary function testing in order to diagnose airway diseases and lung irritation. Laboratory investigations were done in the form of Clara cell protein and immunoglobulin A (IgA) estimation in serum using the immunoassay technique.

Results: According to the thorough occupational history and clinical examination, respiratory system affection was detected among the exposed population in the form of frequent asthmatic attacks, chronic bronchitis and chronic obstructive pulmonary disease. Pulmonary function tests demonstrated marked decrease in the expiratory flow rate 25-75% (FEF_{25-75%}) suggesting irritation and affection of the small airways correlating to duration of exposure more than smoking index. Estimation of the

pneumoprotein CC16 which is produced by the epithelial cells in terminal bronchioles revealed marked elevation in serum of exposed group (149.61 ± 22.64 pg/ml) than among the control group (107.08 ± 12.18 pg/ml). Similarly, the humoral immune defense involving the secretory immunoglobulin A (sIgA) predominant in mucosal secretions was markedly elevated among the exposed than the control groups with mean values of 372.41 ± 232.87 IU/ml and 143.76 ± 83.53 IU/ml, respectively. The serum CC 16 and IgA were both significantly negatively correlated with flow rates. Worth-mentioning, is the increased CC 16 serum levels with ageing which influences the functionality of the lungs and immune system as evidenced by the decline in lung volumes, lung capacity and flow rates and by decreased IgA in serum.

Conclusion: The results suggest that damage of Clara cells in the small airways and the increased epithelial barrier permeability are ultimate consequences of exposure to particulates in air at the workplace. Accordingly, the elevated serum CC16 as a result of increased diffusion of this type of pneumoprotein into the circulation might be used as an early non-invasive biological indicator for lung affection.

Aim of the Work: This study was designed to investigate the cellular and humoral defense mechanisms of the respiratory tract in workers exposed to particulate air pollution in quarries in an attempt to evaluate the importance of using CC16 as a biologic marker for respiratory injury in comparison to pulmonary function tests.

Key words: ambient particulate air pollution, pneumoproteins, Clara cell protein, CC.16, Immunoglobulin A, quarrying, pulmonary function tests.

Introduction

By its nature, quarrying has the potential to create dust and particulate matter (PM) air pollution ⁽¹⁾. In fact, particulate matter air pollution consists of complex and varying mixtures of particles suspended in the air. Of greatest concern to public health are the particles small enough to be inhaled into the deepest parts of the lung. These small particles are known as PM 10 (less than 10 microns in diameter) and even finer particles are known as PN12.5

(less than 2.5 microns in diameter) ⁽²⁾. Accordingly, a large number of quarry workers die a slow death as a result of breaking stone, crushing it and breathing it in as dust ⁽¹⁾.

This ambient particulate air pollution has been associated with adverse respiratory health ⁽³⁾ effects in several studies. Chronic obstructive pulmonary disease (COPD) is the major cause of morbidity and mortality among respiratory diseases. This disorder is characterized functionally

by ⁽⁴⁾ expiratory airflow limitation that is slowly progressive and mainly irreversible . Changes in lung function, reporting of symptoms, hospital admissions or mortality have usually been used as indicators of respiratory disease ⁽⁵⁾ without indication to the presence of biomarkers for lung damage. The mechanisms of adverse effects are still largely unknown. However, inflammatory processes are suspected to play a key role in the pathologic mechanisms starting from deposition of particles to the exacerbation of respiratory diseases ⁽⁶⁾.

The defense mechanisms of the respiratory tract involve both cellular and humoral immune components ⁽⁷⁾. As for the cellular immune defense, prominent ultrastructural characteristics of nonciliated bronchiolar (Clara) cells included an abundance of smooth endoplasmic reticulum and secretory granules ⁽⁸⁾. Analysis of these Clara cells clearly indicated that they synthesize proteinaceous material, Clara cell protein (CC 16), to be secreted from the apical surface into the airway lumen. The nature of Clara cell secretions and the roles played by these secretory proteins in airway homeostasis are only recently being appreciated ⁽⁹⁾.

The Clara cell protein is a 16 kDa lung epithelium specific protein known for the

vulnerability to different insults. Putative roles of CC 16 include an anti-inflammatory effect mediated by inhibition of phospholipase A2 and detoxification of xenobiotics. Recently, it was shown that CC 16 secreted in the respiratory tract diffuses passively across the bronchoalveolar-blood barrier into the serum. Therefore, CC 16 has been proposed to be a useful marker in evaluating the integrity of lung epithelial barrier ⁽⁶⁾.

On the other hand, it is now well accepted that an additional humoral immune defense involving the secretory immunoglobulin A (sIgA) predominant in mucosal secretions, prevents adherence and absorption of noxious bacterial or viral agents. This humoral defense acts as a scavenger through the so-called "immune exclusion". This mechanism requires the expression of a specific receptor for polymeric IgA on the basolateral surface of epithelial cells, thus allows their active transport into the mucosal lumen ⁽⁷⁾. This secretory immune system in close cooperation with the epithelial cells, probably acts in synergy with mucociliary clearance and constitutes the first line of defense of the proximal respiratory tract ⁽¹⁰⁾.

Accordingly, tills study was carried out in order to study the defense mecha-

nisms of the respiratory tract against particulate air pollution in quarries. In addition, an attempt was made to evaluate the use of lung specific proteins as early, non-invasive biologic marker for respiratory injury and its validity over the traditional pulmonary function tests.

Materials and Methods

Study Population:

This work was carried out in silica quarries in Alexandria, El-Agamy District. The study population included 54 individuals divided into exposed and control groups. The exposed group consisted of 29 worker randomly selected from those individuals working in the cutting, carrying and packing of stones. An oral consent was obtained from included subjects. The workers were all male's with age ranging from 28 to 59 years. The duration of exposure to silica respirable dust ranged from 14 to 36 years. The total number of tobacco packs smoked in the number of years known as the smoking index (SI) was chosen as a measure of current tobacco consumption (11). As for the control group, a total of 25 individuals working as food sellers in some markets and as waiters in small coffee shops were chosen from places far from dusty areas, thus excluding the

probability of silica dust exposure. The control population matched the exposed groups in age, sex, social factors and special habits.

Clinical Assessment:

Data collection included a detailed assessment questionnaire interviewing exposed and control groups about the exposure and its pattern. Thorough clinical examination was done for the individuals of both exposed and control groups included in the research for the diagnosis of bronchial asthma, chronic bronchitis and chronic obstructive pulmonary disease (COPD). Diagnosis of COPI) was based on history of chronic bronchitis associated with old reports of decreased FEVI/FVC% below 70% after inhalation of short-acting bronchodilator as well as recently performed pulmonary function tests carried out in the current study (12).

Spirometry:

The spirometric measurements for all individuals were carried out around 3 hours from the beginning of the shift, as the assessment was not feasible, neither before nor after the shift work. The parameters obtained using a calibrated portable vitalograph included the forced expiratory volume in one second (FEV1%); forced

vital capacity (FVC%); forced expiratory volume in one second in relation to forced vital capacity (FEV1/FVC01o); forced expiratory flow rate 25-75% (FEF25-75y) and the forced expiratory flow rate 75-85% (FEF75-N5%). The American Thoracic Society standards for testing were followed and the best of 3 trials for all parameters was obtained. The volumes were recorded in liters and corrected for body temperature atmospheric pressure and saturation with water vapor (BTPS).

Laboratory Investigations:

A small sample of 5 nil venous blood was taken under complete aseptic conditions from all individuals. The sample was kept in a plain tube without anticoagulant for serum separation to determine the immunoglobulin A (sIgA) level in serum. Estimating the sIgA level was done using single radial immunodiffusion plates. Well diameters were read after 24 hours and the corresponding Igs concentration was determined by plotting against calibrators ⁽³¹⁾. Liver functions namely alanine transaminase (ALT) and aspartate transaminase (AST) as well as kidney function tests namely urea and Creatinine were estimated using Hitachi (911) autoanalyser. La Roche Germany supplied the kits and instruments.

Clara Cell Protein (CC 16) Analysis:

The concentration of Clara cell secretory protein 16 (CC16) in serum was determined by a sensitive immunoassay relying on the agglutination of latex particles. The assay uses the rabbit anti-protein I antibody from Dakopatts (Glostrup, Denmark) and as standard the protein purified in the laboratory. To avoid possible interference by complement, rheumatoid factor or chylomicrons, sera were pretreated by heating at 56uC for 30 min and by the addition of polyethylene glycol (16%, vol/vol, 1/1) and trichloroacetic acid (10%, vol/vol, 1/40). After overnight precipitation at 4uC, the serum samples were centrifuged (3,000g for 10 min) and CC16 was determined in the supernatants. All samples were analysed in duplicate at two different dilutions. The validity and the analytical performances of the CC] 6 latex immunoassay (LIA) in different biological media have been reported previously (14,15). The assay has a detection limit of 0.5 mg/L and an average analytical recovery of 95%, with the intra- and inter-assay coefficients of variation ranging from 5 to 10%.

Statistical Analysis.

The mean and standard deviation (SD) were calculated. Unpaired student's t-test

was used to evaluate the relation between the different indices and compare the two study groups. Pearson correlation coefficient was used to relate between age, duration of exposure, smoking index, levels of immunoglobulin A (sIgA) and clara cell protein (CC16) in serum, as well as the different parameters of pulmonary function tests. P value of <0.05 was considered significant.

Results

This study was carried out on a group of workers exposed to respirable and non-respirable dust particles in stone quarrying. The control group was selected so as to match the exposed group in age, sex, social conditions and smoking habits. The mean \pm SD of age was 48.69 ± 7.82 year in the exposed group and 46.88 ± 6.34 year in the control group. As regards the smoking index, the mean \pm SD were 18.28 ± 7.77 and 19.72 ± 6.18 among the exposed group (n=29) and the control group (n=25), respectively. The duration of exposure for the exposed population ranged from 14 to 36 years with a mean of 26.07 ± 6.36 year.

The frequency distribution of the most important relevant clinical manifestations among the exposed workers and the control group were evaluated, data not pre-

sented. According to the thorough occupational history and clinical examination, respiratory system affection in the form of harsh vesicular breathing (21 cases; 72.4%) and chest wheezes (24 cases; 82.8%) are commonly present among the exposed population compared to 3 cases (12%) and 2 cases (8%) among the control group, respectively. Additionally, frequent asthmatic attacks were reported among 10 (34.5%) of the exposed workers, whereas only 2 (8%) of the unexposed workers complained of attacks of asthma. Chronic bronchitis, defined by presence of productive cough for three consecutive months for at least 2 years, was diagnosed in 22 (75.9%) exposed worker in contrast to only 2 (8%) among the control group. A total of 22 cases (75.9%) suffering from COPD were detected among the exposed population and only 2 cases (8%) among the control group. No statistically significant difference was detected among both groups as regards the clinical data.

Estimation of clara cell protein (CC16) in the study population revealed marked elevation of the protein in the serum of exposed group with a mean of 149.61 ± 22.64 pg/ml, than among the control group (107.08 ± 12.18 pg/ml). Similarly, the level of immunoglobulin A in serum (sIgA) was higher among exposed than control

groups; mean values being 372.41 ± 232.87 IU/ml and 143.76 ± 83.53 IU/ml, respectively. The difference in the levels of CC] 6 and sIgA between both groups proved to be statistically significant, P value < 0.05 . Evaluation of the liver and kidney functions was carried out to assess the deleterious effects of silica on both liver and kidney function. Among the liver functions, serum glutamic-oxaloacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT) enzymes were slightly elevated above normal, but no statistical difference was obtained between the exposed (n=29) and control (n=25) groups. Similarly, the kidney functions revealed no statistically significant difference between the groups (Table 1).

The pulmonary function tests were carried out for both exposed and control groups. The data obtained revealed no significant difference between the results of all parameters except for the forced expiratory flow rate 25-75% (FEF25-75) which showed a statistically significant difference between the groups. The mean values obtained were 55.79 ± 21.85 among the exposed group and 83.56 ± 8.43 for the control group (Table 2).

Further assessment of the relationships between the different variables in the study was done. Accordingly, a negative correla-

tion was revealed for the effect of age versus both the pulmonary function tests and sIgA level, while, a positive correlation was detected versus the CC16 serum level. All associations were not statistically significant except for FEF25-75% rate as the association proved to be highly significant (P value < 0.005). Additionally, a highly significant negative correlation was obtained between the effect of smoking (SI) and both CC 16 and FEVI/FVC. Studying the association between the duration of exposure to dust particles and different parameters showed a negative correlation that proved to be highly significant for FEF25-75% rate but only significant for FEF75-85% rate (Table 3).

The levels of serum clara cell protein and immunoglobulin A subsequently inter-correlated revealed a positive highly significant association in between the two parameters. As regards the relation between the Clara cell protein and pulmonary function parameters, a significant negative correlation was obtained for FVC and a positive correlation for FEVI. The IgA showed a significant negative correlation with FEVI/FVC and a highly significant correlation with FVC. Additionally, highly significant negative relations were revealed for both CC 16 and IgA versus the flow rates (Table 3).

Table (1): Mean \pm SD of the results of Different Laboratory Investigations among the Group of Workers exposed to Silica Dust in Quarries (n=29) and the Non-exposed Control Group (n=25).

		Exposed Group n = 29	Control Group n = 25	t-test	(p-value)
Urea	Range	20.2 - 53.2	20.2 - 43.7	0.1522	0.773
	Mean	38.169	35.388		
	SD	6.560	7.505		
Creatinine	Range	0.82 - 2.04	0.7 - 1.9	0.1736	
	Mean	1.320	1.184		
	SD	0.375	0.346		
ALT	Range	5.7 - 56.3	6.7 - 34.6	0.0339	0.0001*
	Mean	26.910	19.78		
	SD	14.079	8.959		
AST	Range	14 - 56.2	13.8 - 43.7	0.0079	0.0001*
	Mean	34.328	25.992		
	SD	12.445	9.158		
CC16	Range	98.7-196.2	85.8 -124.8	2.500	0.0001*
	Mean	149.614	107.084		
	SD	22.464	12.176		
sIgA	Range	80 - 920	56 - 451	2.280	0.0001*
	Mean	372.414	143.76		
	SD	232.865	83.531		

Urea (mg/dl); Creatinine (mg/dl); ALT: Alanine Transaminase (IU/L); AST: Aspartate Transaminase (IU/L);

CC16: Clara Cell Protein 16 (pg/ml); sIgA: serum Immunoglobulin A (IU/ml).

NS: Non-significant $P > 0.05$ * Significant $P < 0.05$

Table (2): Mean \pm SD of the results of Pulmonary Function Tests among the Group of Workers exposed to Silica Dust in Quarries (n=29) and the Non-exposed Control Group (n=25).

		Exposed Group n = 29	Control Group n = 25	t-test	P value
				0.0386	N.S.
FVC%	Range	39 - 98	72 - 99		
FEV1%	Mean	76.379	85.120	0.0176	
FEV1/FVC%	SD	19.301	7.661		N.S.
FEF25-75%	Range	46 - 103	65 - 96	0.0218	
FEF75-85%	Mean	88.862	81.360		N.S.
	SD	13.330	8.046	2.0378	
	Range	42 - 98	55 - 99		0.0467*
	Mean	70.759	79.840	1.2306	
	SD	14.111	14.019		N.S.
	Range	17 - 99	65 - 99		
	Mean	55.789	83.560		
	SD	21.845	8.431		
	Range	18 - 99	60- 101		
	Mean	53.966	80.240		
	SD	20.547	12.451		

FEV1%: Forced Expiratory Volume in one second; FVC: Forced vital capacity;

FEV1/FVC: Forced Expiratory Volume in one second in relation to Forced vital capacity;

FEF25-75%: Forced Expiratory Flow rate 25-75%; FEF75-85%: Forced Expiratory

Discussion

The particulate matter (PM) emissions from quarries can cause adverse effects to neighboring communities and to the environment. Quarry dust generated comes from a number of different activities and sources including crushing and screening (50%), haul roads and traffic (48%) and to a lesser extent from blasting (2%) (16). Bench and block cutting is a wet process, with minimal exposure to fine dust. Nevertheless, when mud dries on the quarry floor, workers are exposed to the inhalation of dust raised by wind and the transit of the vehicles (17).

It is only the very smallest particulate matter (PM_{2.5}) which is only 1-2 pm that is transported into the alveoli and which actually leads to chronic health effects. With regard to particle size, a recent report calculates that only 28% of all dust generated in quarrying is respirable, most of the dust emitted is in the coarse size range, more than 2.5 μm. The risk of silicosis depends on the amount of quartz actually present in the dust and the diameter of free silica particles. In many work operations, the exposure level to respirable dust in Egypt can reach a daily average 400 Rg/m³ exceeding the Air Quality Limit Value 70 Rg/m³ for Egypt (19). These high

concentrations are observed near the areas of brick industries and quarries as well as crowded and traffic-congested roads (20).

Quarry workers are affected not only because of the dust content of crystalline silica, but also because of exposure to unfavorable macroclimatic conditions, and tobacco smoking (17). Yet, exposure to particulate air pollution is the major factor for increasing epithelial barrier permeability in the lung leading to chronic obstructive pulmonary diseases (COPD) (21).

In patients with severe COPD, secretory immunoglobulin A (sIgA)-dependent immunity was found to be clearly impaired in the bronchi (14). The sIgA was thought to prevent chronic mucosal inflammation by down-regulating the pro-inflammatory processes mediated through monocytes and neutrophils (22), and by inhibiting adherence of antigens and microorganisms to the surface epithelium (7). Impairment of secretory immunity was found to provide a link between structural changes and inflammation (23).

Recently, in addition to the humoral sIgA-dependent immunity, a cellular type of immunity at the level of the epithelial barrier was considered. In small airways, the main epithelial cell type constituting

the Clara cells were found to play an important physiological role in surfactant production, protection against environmental agents, in regulation of inflammatory and immune responses in the respiratory system and to be involved in lung homeostasis (24). Additionally, these epithelial cells are altered in diseases like asthma, Chronic Obstructive Pulmonary Diseases (COPD), cancers and several pulmonary pathological processes induced by various lung toxicants, thus secreting a specific type of protein. Clara cell protein (CC16) produced is a 15.8-kDa protein secreted all along the tracheobronchial tree and especially in the terminal bronchioles where Clara cells are localized (25).

Being small in size, CC16 secreted in the distal air spaces, readily diffuses into the plasma. Several lines of evidence suggest that proteins cross the air-blood barrier paracellularly, through water-filled pores located at the junctional complexes linking epithelial and endothelial cells (26). CC16 entering the circulation distributes in the vascular and extravascular compartments and is rapidly eliminated by the kidney (27).

In this study, a group of workers exposed to respirable and non-respirable dust particles in stone quarrying were randomly

selected. Occupational exposures to respirable silica-containing stone dust were found to be associated with the development of silicosis, lung cancer, pulmonary tuberculosis, and airway diseases. These exposures were also related to the development of autoimmune disorders, chronic renal disease and other adverse health effects (28).

In the current study, assessment of kidney and liver functions for the studied population showed no elevation or above normal levels for both urea and creatinine or for liver enzymes. However, earlier epidemiologic studies found statistically significant associations between the occupational exposure to PM 2.5 crystalline silica dust and several renal diseases or effects ranging from subclinical renal changes (29) to end-stage renal disease caused by glomerulonephritis (30). Some case reports provided evidence of an immunologic injury by immune complex formation, and other reports pointed to a direct toxic effect of PM content of silica (31). In workers exposed to crystalline silica, hepatic changes and hepatocellular carcinoma have been identified, though the cause-effect relationship was not clear (32).

Ambient air particulate pollution has been associated with adverse respiratory health effects in several studies (3). There

is growing recognition that occupational exposures make a substantive contribution to adult-onset asthma, chronic bronchitis and other respiratory symptoms ⁽³³⁾. Chronic obstructive pulmonary disease (COPD) is the major cause of morbidity and mortality among respiratory diseases. Physiologic investigations have indicated that small airways are a major site of increased resistance in COPD ⁽³⁴⁾. This disorder is characterized functionally by expiratory airflow limitation that is slowly progressive and mainly irreversible ⁽⁴⁾.

Although cigarette smoking is well documented as the major risk factor for COPD, only a minority (15 to 20%) of heavy smokers will develop COPD ⁽¹⁶⁾. In this study, the disease was diagnosed among 22 (75.9%) of exposed workers compared to 2 (8%) cases of the control group, and the difference was not statistically significant. The disease detected among the control group might have been attributed to cigarette smoke, as both cases were moderate smokers with smoking index 26 and 28 pack-year, respectively.

There is now growing evidence that airways obstruction (COPD) are caused by occupational exposures to PM_{2.5}. Most of these workers started working in the field very early in life with mean value of expo-

sure onset around 22.62 ± 4.7 years. In fact, age-related structural and functional changes in the lung have been previously described. Gas trapping increases with age as do airspace abnormalities and diameter, whereas lung compliance decreases in association with increased lung elastin content ⁽³⁵⁾. Moreover, the internal surface area of the lung increases to a maximum of 80 m² at the age of 20 years, but decreases by 2.7 m² per decade thereafter [36], therefore the exposure in early adult life appeared to have more effect than later exposure ⁽³⁷⁾.

The duration of exposure to dust particles in the current study that ranged from 14 to 36 years (mean value 26.1 ± 6.4 years) was associated with affection of 13 (44.8%) cases from airflow obstruction and chronic obstructive pulmonary disease as diagnosed based on history and lung function tests ⁽¹²⁾. Severe affection of the expiratory flow rates was correlating with the duration of exposure, in contrast to the other parameters which were not markedly if at all affected. Many studies with quantitative data on silica dust exposure reported statistically significant associations between loss of lung function and cumulative respirable dust exposure in workers [38]. Exposure to dust containing 0.09 mg/

m³ of crystalline silica at an average respirable concentration of 0.3 mg/m³ for 24 years was associated with loss of 236 ml of FEV₁. This loss was reported to be equivalent to about half of the estimated loss of FEV₁ in a typical male who smoked one pack of cigarettes per day for 30 years (552 ml) (39). No significant difference in smoking index (SI) was reported between both groups included in the present study, yet correlating the data among the whole study population showed a negative highly significant association of SI versus the FEV₁/FVC. In fact, the combined effects of respirable dust exposure and smoking on the loss of FEV₁ must be additive (40).

Though lung function tests alone cannot diagnose any particular disease (41), yet they are an important part of the clinical evaluation of workers with occupational diseases. They are not diagnostic for silica exposure and silicosis (42). Currently there are no biological markers sufficiently sensitive to assess parenchymal lung health before functional abnormalities become apparent (43).

The use of induced sputum, nasal lavage, or the measurement of exhaled gases and breath condensate has been championed by many as providing less invasive

methods for assessing pulmonary responses to air pollution challenges (44). In contrast, these methods have failed to demonstrate increased permeability in these studies. Tests based on the appearance of lung-specific proteins in serum following an airway insult have been proposed as a noninvasive and highly sensitive alternative to traditional markers of lung epithelial injury (45).

These proteins occur physiologically in small amounts in blood (46), being synthesized and secreted by the lung Clara cells. Other sex-linked sources such as the uterus and the prostate contribute nothing or very little to the serum CC16 concentrations (47). However, serum concentrations of CC16 show considerable variations in healthy subjects, the baseline concentrations reflect the number of Clara cells whereas the variation in time reflects the integrity of the lung epithelial barrier. This study was able to demonstrate a negative highly significant correlation between the level of CC16 and the smoking index. Tobacco smoke was able to induce reduction in CC16 serum levels due to decreased density of Clara cells in the lungs (48). Since Clara cells have a high content of xenobiotic metabolising enzymes, they are probably progressively destroyed by toxic

metabolites of tobacco smoke generated via the cytochrome P450 system and might be involved in the progressive destruction of lung parenchyma ⁽⁶⁾.

However, serum concentration of CC16 has been reported to increase slightly with aging ⁽⁶⁾. In fact, a positive correlation was determined with age among the studied population but was non-significant. The Clara cell protein produced in large amounts into the lumen of the respiratory tract, a condition referred to as pneumoproteinaemia, cross the lung epithelial barrier into the vascular compartment through passive diffusion ⁽⁴³⁾. Although the mechanism for the chronic increase in leakage is unknown, recent findings suggest that the changes are associated with repeated inflammatory-mediated acute lung injury, increased oxidant load and depletion of antioxidants including glutathione ⁽⁶⁾.

Indeed, their intravascular leakage increases in conditions characterized by pulmonary inflammation and/or pulmonary epithelial injury ⁽⁴⁶⁾. Degranulation and consequent secretion of granule contents can be induced by inhaled pollutants [49] in several situations known to be associated with a disruption of the air-blood barrier, such as pulmonary fibrosis, interstitial

lung diseases and in lung injury caused by lung irritants ⁽⁴⁵⁾.

Airway irritation caused by particulate air pollution is known to impair lung function and induce airway inflammation ⁽⁵⁰⁾. Previous studies have failed to find a clear association between lung function decrements and airway inflammation ⁽⁵¹⁾. It has been suggested that lung function decrements are related to stimulation of airway C-fibres ⁽⁵²⁾ and to the release of substance P from sensory nerves associated with epithelial injury. A significant reduction in FEV1 was observed immediately post-exposure, at this time serum CC16 concentrations were not increased suggesting that these two responses are not simply associated ⁽⁵³⁾.

On the contrary, studying the association between the pulmonary function tests and the level of CC16 revealed highly significant negative results with the expiratory flow rates but significant with functional vital capacity indicating the presence of a restriction element as a result of accumulating proteins. As for the other pulmonary function parameters, a positive correlation was obtained, proved to be significant with the FEV1, but non-significant with FEV1/VC. In fact, previous epidemiological studies reported that subjects with chronic

obstructive pulmonary disease have a significant reduction of CC16 in serum, pneumoproteinaemia inversely correlating with FEV1/VC (43), and that mid-expiratory flow rates are powerful predictors of mortality from COPD (54).

However, the functional roles of Clara cell secretions that are induced by exposure to various environmental agents are poorly understood. Despite the paucity of information regarding biogenesis and packaging of secreted proteins from Clara cells, considerably more work has been performed investigating mechanisms of Clara cell secretion. A number of studies have demonstrated that secretion of granule contents by Clara cells is activated by either adrenergic or cholinergic agents (55). Clara cell secretory protein has been shown to interact with microsomal and plasma membranes through binding to an integral membrane protein (56), and has been shown to bind other secreted proteins such as fibronectin (57). Lack of these interactions may result in alterations in the signaling events which contribute to the synchronization of protective responses, such as the degranulation of Clara cells and subsequent activation of epithelial regeneration (58). As such, Clara cell protein has been ascribed an anti-inflammatory

role in lung diseases (59).

In chronic obstructive pulmonary disease, bronchial and parenchymal damage is thought to lead to defects in airflow and gas exchange. Despite important epithelial changes in the bronchi, only indirect data suggest a decreased production of epithelial-derived CC16 that is more striking in small than in large airways (60). The decreased expression of CC16 is proved by the results of pulmonary function tests performed on the current studied population which revealed a highly significant inverse reduction of the expiratory flow rates 25-75% and 75-85% with the CC16 concentrations. This finding is due to the relative preservation of CC16 expression in large airways as a result of goblet cell hyperplasia which is more intense in these airways (61). These studies indicate that modulation of Clara cell secretory function may play an important role in airway homeostasis and disease pathogenesis. Clara cell secretory protein has been described as a "multifunctional protein" and among these many postulated functions is most frequently quoted as being a potent regulator of the inflammatory response (62).

The relationship of inflammatory and immune parameters to structural changes in the airways remains unclear. It is now

well accepted that the secretory immunoglobulin A (SIgA), the predominant Ig isotype in mucosal secretions, prevents adherence and absorption of noxious agents, acting as a scavenger through so-called "immune exclusion". The IgA is produced by the mucosal subepithelial plasma cells in dimeric form which is actively transported across the airway epithelium by trans-cellular routing assumed by the polymeric immunoglobulin receptor (pIgR). At the apical pole of the airway epithelial cell, the IgA-pIgR complex is cleaved from the cell membrane to release the secretory IgA, which is formed by dimeric IgA covalently bound to the extracellular part of the pIgR, called the secretory component (SC) (7).

Estimation of the sIgA in serum of the study population revealed significant elevation not correlating with age, smoking index or the duration of exposure, but correlating positively and highly significantly with the level of CC16 in serum. Experimental studies revealed that the secretory immune system implies close cooperation between the submucosal immunoglobulin-secreting plasma cells and the epithelial cells, thus acting in synergy with the mucociliary clearance and constituting the first line of defense of the proximal respir-

atory tract (63). In addition to the positive correlation with CC16, a negative highly significant correlation between the IgA and expiratory flow rates as well as the FVC and FEV1/FVC was revealed. Related studies reported that deficiency of CC16 and altered expression of immunoglobulin A may be associated with global alterations in lung homeostasis with either direct or indirect effects on lung immunoregulation (63).

Studies carried out recently proved that the expression of the pIgR is decreased in patients with mucosal diseases such as chronic inflammatory diseases of the airways caused by the exposure to air particulates. These results were strongly demonstrated in the bronchial epithelium of patients with severe COPD but not in smokers without COPD. Moreover, pIgR expression correlated with lung function tests of airway obstruction such as the forced expiratory volume in one second (FEV1). Therefore, the impairment of bronchial secretory immunity was suggested to play a role in COPD pathogenesis following exposure to low-dose endotoxin (64).

The IgA is therefore the main antibody isotype in mucosal secretions of the respiratory tract, thus contributing to frontline

mechanisms of defense along with mucociliary clearance and innate epithelial-derived factors. However, the role of IgA in chronic airway inflammation and whether it represents a consequence of the disease or is implicated as a driving factor of its development, playing a causative role and presumably related to intrinsic changes of bronchial epithelium is to be evaluated.

Nevertheless, the utility of epithelial cells and the secreted Clara cell protein 16 in serum that reliably reflects the leakage of the lung epithelial barrier to proteins, appear to be useful indices, integrating components of both cellular toxicity and epithelial barrier function. Therefore, the CC16 might be proposed a useful marker of airway and lung epithelial injury. Potentially, the concept of pneumoproteinaemia has the equivalent utility to that of monitoring proteinuria in kidney diseases, since the passage of proteins across the lung barrier is governed by similar structural and functional features as that of glomerular filtration.

Consequently, the use of different biomarkers, produced by distinct regions of the respiratory tract, may allow evaluation of present and past exposures to toxins, detection of early defects, identification of

susceptible individuals and the derivation of acceptable exposure levels, thus helping establish causal associations between effects and putative toxic agents. However, the application of serum CC16 as a noninvasive biomarker for air pollution-induced lung epithelial injury should be considered with caution being related to potential diurnal variation in serum concentrations, the impact of background exposures on CC16 responses and the large inter-subject variation in baseline concentrations. Larger studies are, therefore, needed to determine normal variance before utility of pneumoproteinaemia can be fully exploited. Despite these limitations, the strength of the data further underpins potential utility of pneumoproteins as markers of parenchymal lung health.

Results obtained from this study underlines the importance of carefully standardized studies in air pollution epidemiology and the utility of efforts to decrease concentration of ambient air pollutants as harmful health effects are observed even at low concentrations. Accordingly, a prevention plan for the protection of workers in quarries should be addressed.

Periodical inspections of the workplace and the exposed workers are needed with the subsequent removal of affected

personnel. Collective and individual preventive measures should be adopted, together with the implementation of medical surveillance programs that provide clinical evaluation at least once a year. In addition to health history and physical examination, specific tests are to be carried out every one or two years in the form of pulmonary function tests and chest radiography in quarrymen exposed to dust and estimation of CC16 for the early detection of lung irritation in an attempt to detect lung affection before the disease is irreversible.

To reduce exposure to inhalable dust, prevention measures should include scrupulous wet cutting, cleaning of the work area by clearing away the mud by hand or with a mini-loader, and wetting the quarry floor and the access roads, especially in the drier seasons. During these operations, workers have to wear adequate personal protective equipment to protect respiratory airways and lungs. Underground sites have to be equipped with ventilation systems, adjustable according to the advancement of the exploitation in addition to increase in the natural ventilation by adequately widening the emergency exits or by creating special openings.

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Table-2: Chi-square test (Number and percentage) of persons having and not having recurrent infections, sensory & motor manifestations, cranial nerve involvement, manifestations of increased intracranial tension and impotence in group I and II as well as their statistical significance.

Variable	Group	Number & Percentage of persons having	Number & Percentage of persons not having	Statistical significance (p-value)
Recurrent infections	Group I	- (0%)	20 (100%)	0.0001*
	Group II	14 (63.64%)	8 (36.36%)	
Sensory affection	Group I	- (0%)	20 (100%)	0.139
	Group II	8 (36.36%)	14 (63.64%)	
Motor affection	Group I	- (0%)	20 (100%)	0.0001*
	Group II	16 (72.73%)	6 (27.27%)	
Cranial nerve affection	Group I	- (0%)	20 (100%)	0.037*
	Group II	6 (27.27%)	16 (72.73%)	
increased intracranial tension	Group I	- (0%)	20 (100%)	0.0001*
	Group II	14 (63.64%)	8 (36.36%)	
Impotence	Group I	- (0%)	20 (100%)	0.037*
	Group II	6 (27.27%)	16 (72.73%)	

* = significant difference

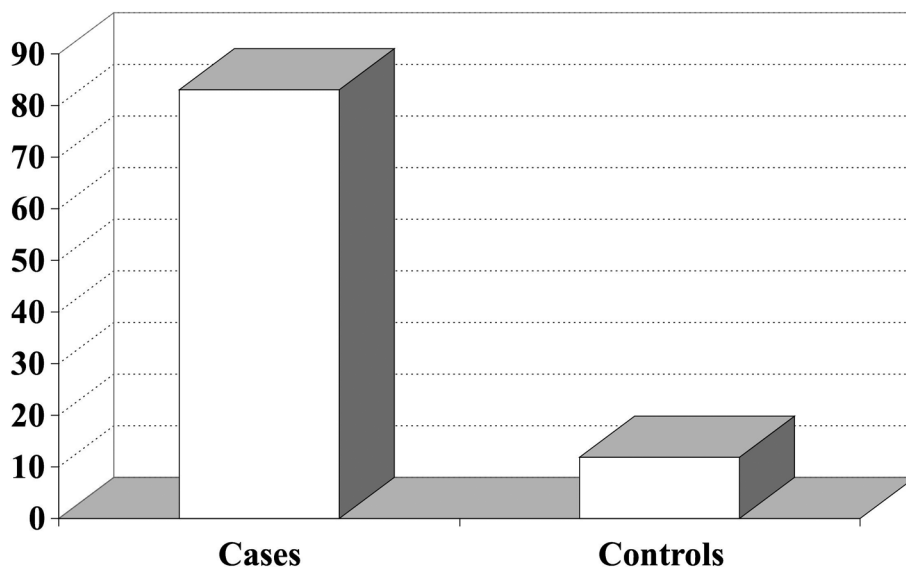


Figure (1): Mean values of blood lead ($\mu\text{g/dl}$) in cases and controls.

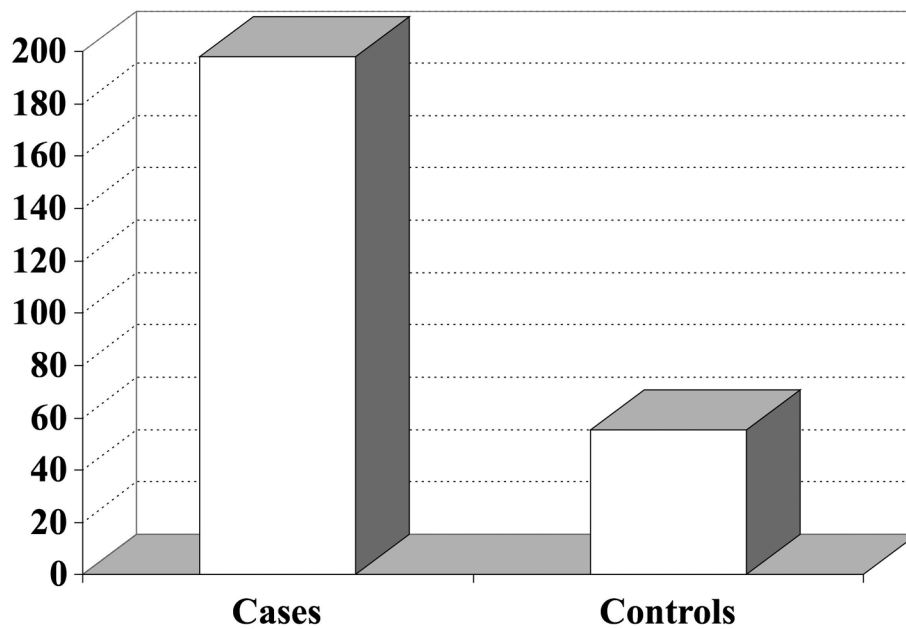


Figure (2): Mean values of serum MMP ($\mu\text{g/ml}$) in cases and controls.

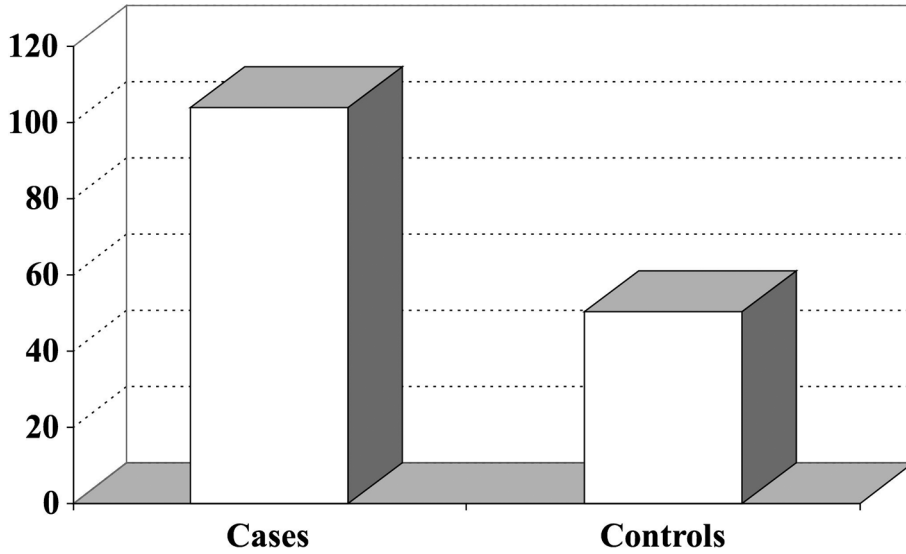


Figure (3): Mean values of serum TNF α (pg/dl) in cases and controls.

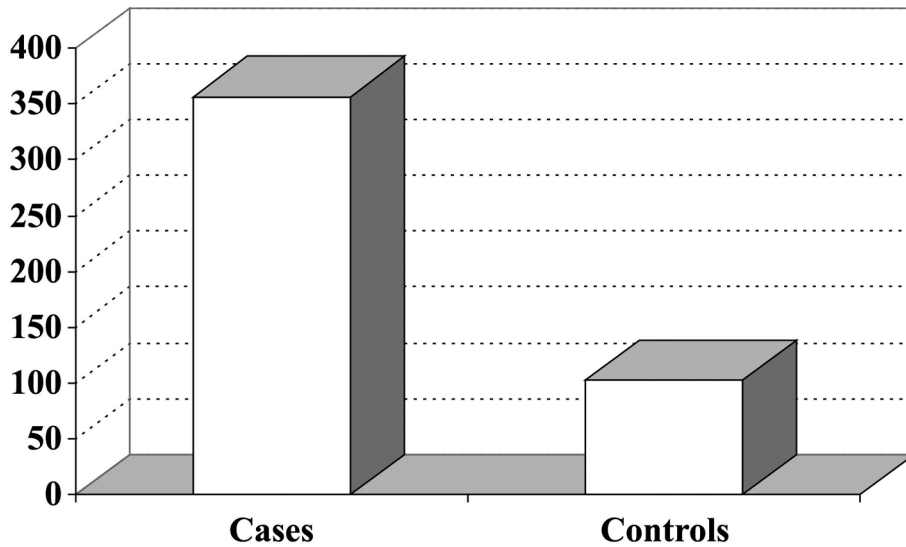


Figure (4): Mean values of serum IL1 (ng/dl) in cases and controls.