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# The Monitoring of Serum and Urine heavy Metals and assessment of inflammatory response, Respiratory Symptoms, and Pulmonary health in Cement-Exposed Workers

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## ABSTRACT

Air pollution in the workplace is a major health issue in developing countries. Cement manufacturing is a complex process that extracted the raw materials to the grinding of the end product in the cement factories (CF). Workers in CF are exposed to many harmful factors, such as dust and respirable particles of cement. This study is aimed to compare the respiratory symptoms, pulmonary function tests (PFT), total and differential white blood cells (WBC), serum level of high-sensitivity C-reactive protein (hs-CRP), and serum or urine heavy metal levels were between CF workers with control subjects.
The case-control study with convenience sampling was undertaken. A total of 40 non-smoking male cement workers with 40 healthy volunteers were recruited in the study. Respiratory symptoms, pulmonary function tests (PFT), total and differential white blood cells (WBC), serum level of high-sensitivity C-reactive protein (hs-CRP), and serum or urine heavy metal levels were compared between the two groups.
The frequency of respiratory symptoms including wheezing, dyspnoea, cough, and sputum, haematological parameters, serum level of hs-CRP level, as well as serum or urine heavy metal levels were significantly higher in cement workers. All PFT values except forced vital capacity (FVC) were lower in workers of the CF workers compared to control subjects.
Respiratory insult, systemic inflammation and heavy metal changes were shown in CF workers. Increased serum and urine heavy metals may induce inflammatory processes leading to lung disorders. These results emphasize that the continuous monitoring of workers' workplace safety and occupational status.

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## INTRODUCTION

Currently, developing countries try to provide safe disposal of cement kiln dust for the production of new materials such as geopolymers (Abbas *et al*., 2021). In addition, recycling of solid-waste materials could help to reduce raw material consumption and CO2 emissions (Abdelzaher, 2023). The recycling system can reduce the production of environmental

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pollutants due to reduced energy consumption which showed a low risk in the environmental impact assessment (EIA) study (Abdelzaher *et al*., 2023).

Air pollution has long been a health threat to humans and a serious concern for clinicians and lawmakers. Among the most important sources of pollution are industrial factories such as cement factories (CFs), releasing manifold fine particles into the air, which induced a serious health threat to the workers and nearby residents (Parvin *et al*., 2020). Increased prevalence of dyspnoea, chronic phlegm, and wheezing were reported in CF workers (Neghab & Choobineh, 2007). The chest manifestations of one-third of Egyptian cement workers, was shown along with increased urinary creatinine, plasma malondialdehyde (MDA), and p53 expression levels bud decreased total thiol levels which were associated with increased blood chromium (Elhosary *et al*., 2014). In exposing workers to cement dust, increased cough, phlegm, wheeze, dyspnea, bronchitis, sinusitis, shortness of breath and bronchial asthma, and decreased pulmonary function tests (PFT) were also reported (AminianAslani & Sadeghniiat Haghighi, 2012). In Tanzanian CF workers also increased chronic cough, sputum production, dyspnea, shortness of breath, COPD, and chronic bronchitis were shown which were related to cumulative cement dust exposure (Mwaiselage *et al*., 2006).

Workers in the cement production factories are in contact with dust containing toxic metals such as nickel, cobalt, lead, cadmium, manganese, and chrome, which are water-soluble and can pass the blood-respiratory barrier, causing pulmonary complications. Heavy metals and other toxic agents in the CFs can lead to oxidative stress and inflammatory disorders. Moreover, since the number of these workers is rising as the world becomes more industrialized, more and more people will have occupational exposed cement (OEC), (Abolhasannejad *et al*., 2010; Sultan A Meo, 2004).

Cement dust contains crystalline silica, of which quartz is the most common form, and is the second most common mineral on the planet's surface. Each of the three major types of rocks (igneous, metamorphic and sedimentary) contains quartz. Chromium is another heavy metal found in cement dust and its various oxidant states (Cr II-Cr IV) is a common building block for many different compounds. To make chromium metal and chromates, chromium is extracted from the earth (III) (Ahmad *et al*., 2021; Richard *et al*., 2016; Shanshal & Al-Qazaz, 2021).

Increased level of Cadmium, Cobalt, Chromium, and Nickel in the floating particulates of CF environment was shown (Abolhasannejad *et al*., 2010). In Norwegian cement production workers, values of FEV1, FEF25-75%. DLCO and IL-10 were decreased but FeNO, white blood cell (WBC) count, fibrinogen, and TNF-α levels were increased (Fell *et al*., 2011). In addition, increased neutrophils and IL-1β levels in the sputum of cement production workers in the exposed period compared to the unexposed period were reported (Hakim *et al*., 2018). In cement workers, decreased phagocytic activity of polymorphonuclear neutrophils (PMNs) was also demonstrated (Sultan Ayoub Meo *et al*., 2008).

Although the significant association of OEC with reduced respiratory capacities and induction of respiratory symptoms has been demonstrated by some studies, other studies did not report associations likewise, and more studies are required to clarify this issue. In addition, there is limited information regarding the harmful effects of cement dust on CF workers. Therefore, the present study was performed to assess the association of OEC with respiratory disorder in CF workers in Mashhad, Iran by measuring heavy metal blood and urine levels, blood inflammatory cells and mediators, pulmonary function tests, and self-reported respiratory symptoms.

## MATERIALS AND METHODS

### *Study design and Participant Recruitment*

In this case-control study, 80 men, including 40 cement-exposed workers from an OPC factory (with the composition described in Table 1) and 40 healthy control subjects who were

**Table 1.** Various compositions of Ordinary Portland Cement

| Name | Chemical Structure | Percent |
|---|---|---|
| **Calcium carbonate** | $CaCO_3$ | 60-67 |
| **Silicon dioxide** | $SiO_2$ | 17-25 |
| **Aluminium oxide** | $Al_2O_3$ | 3-8 |
| **Ferric oxide** | $Fe_2o_3$ | 1-5 |
| **Magnesium oxide** | MgO | 1-5 |
| **Sodium oxide and Potassium oxide** | $Na_2O+K_2O$ | 5/1-1 |
| **Sulfur trioxide** | $SO_3$ | 1-3 |

**Table 2.** The criteria for respiratory symptom severity score

| Symptoms | Frequency | Score |
|---|---|---|
| **Chest wheezing** | None | 0 |
| | Hardly heard wheezing | 1 |
| | Moderate wheezing | 2 |
| | Loud wheezing | 3 |
| **Night cough** | None | 0 |
| | Good sleep with a little cough | 1 |
| | Awake up from sleep (one time) | 2 |
| | Awake up from sleep (more than one time) | 3 |
| **Night wheezing** | None | 0 |
| | Good sleep with a little cough | 1 |
| | Awake up from sleep (one time) | 2 |
| | Awake up from sleep (more than one time) | 3 |
| **Cough and wheezing due to exercise** | None | 0 |
| | During heavy exercise | 1 |
| | During mild exercise (walking) | 2 |
| | At rest | 3 |

referred to a medical diagnostic laboratory in the Mashhad city (Iran) for routine check-up tests were studied. All control subjects' living and working places were far away from the CF. The control subjects were matched for age, sex, and demographic factors with the subjects of the case group. All workers worked 8 hours a day for 6 consecutive days each week. All participants were non-smoker men who did not report any history of respiratory infection, other respiratory diseases, or thoracic surgery before cement exposure. Informed written consent was obtained from all the subjects.

*Measurement of blood and urine heavy metal levels*

Using the standard technique, blood samples of 3-4 ml were gathered into plastic syringes. One ml of each sample was placed in a K3EDTA-containing plastic tube and stored at 4°C for the measurement of serum lead and cadmium concentrations.

Fresh urine samples of about 50 ml were transferred into a metal-free plastic container and acidified by adding 200 µl of nitric acid. Then the urine samples were kept frozen for further analysis of nickel, cobalt, and chromium concentrations.

The samples were analyzed for heavy metal concentrations using an atomic absorption spectrometer (Model 3030, Perkin Elmer, United States) with a cement hollow cathode lamp (HCL) and electrodeless discharge lamp (EDL) and a heated graphite atomizer (HGA)-400 graphite furnace with deuterium background corrector. Standard solutions with predefined concentrations of the measured elements were used initially to calibrate the data based on the traceable certified reference materials of the National Institute of Standards and Technology (NIST). The measured heavy metal in the blood and urine were selected according previous studies (Owonikoko *et al*., 2021; Yahaya *et al*., 2022; Zhang *et al*., 2023).

*Measurement of haematological and inflammatory markers*

The remainder of each blood sample was placed into another K3EDTA-containing plastic tube and immediately centrifuged at 800 rev/min for 15 minutes. The separated plasma was stored at -20°C for subsequent hs-CRP measurement.

All the samples also analysed for complete blood count (CBC). In the CBC test, total red blood cell (RBC), white blood cell (WBC), and platelet (PLT) counts were measured. In addition, the percentage of each WBC type, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils, were also calculated. Furthermore, the levels of RBC indices, including haemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), MCH concentration (MCHC), and RBC distribution width (RDW) were measured. Lastly, platelet indices, including mean platelet volume (MPV) and platelet distribution width (PDW), were also measured.

*Evaluation of respiratory symptoms and PFT values*

A questionnaire for respiratory symptoms based on the previous studies (M. H. Boskabady *et al*., 2007; Hashemi *et al*., 2010; Khazdair *et al*., 2012) was used to assess the prevalence and severity of respiratory symptoms, including wheezing, dyspnea, cough, and sputum (Table 2). Medical history, including atopy and history of allergic reactions, was also obtained from the participants.

Pulmonary function tests (PFT) were assessed by an expert operator with standard techniques and conditions recommended by the American Thoracic Society (ATS, 1995). Using a spirometer with a pneumotachograph sensor (Model ST90, Fukuda, Sangyo Co., Japan), PFT was performed three times for each subject, and the highest scores for forced vital capacity (FVC), forced expiratory volume in one second (FEV1), peak expiratory flow (PEF), maximum mid-expiratory flow (MMEF) and maximum expiratory flow at 75%, 50%, and 25% of the FVC (MEF75, MEF50, and MEF25, respectively) were recorded. The value of spirometry parameters was calculated as a percent of the predicted value, based on the age, race, and height standards of the subjects.

*Statistical analysis*

Using the probability proportional to size (PPS) sampling method, it was calculated that a minimum of 40 subjects in each group was needed with an $\alpha$ error of 0.05 and a power of 80%. SPSS software (version 22 for Windows, IBM Statistics, IL, United States) was used to analysed the data. In addition, the sample size of this study is similar to previous similar studies (Fell *et al*., 2010; Kakooei *et al*., 2012; Richard *et al*., 2016). Kolmogorov-Smirnov test was used to assess the normal distribution of the data. Independent samples T-test was used for data analyse. Data were presented as mean ± SEM. *P* values lower than 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

*Characteristics of studied subjects*

Overall, 80 men with a mean age of 37.19 ± 9.31 years were studied, of whom 40 (50%) were workers from a CF, and the remaining 40 were healthy control subjects. The two groups had no significant difference in age and height. Table 3 shows the demographic characteristics of the studied subjects.

*Blood and urine heavy metal levels*

Serum levels of lead and urine levels of heavy metals, including cobalt, nickel, and chromium, were significantly higher in the workers compared to the control individuals ($p < 0.05$ to $p < 0.001$). However, the serum level of cadmium was not significantly different between the two groups (Fig 1 A-C).

**Table 3.** Demographic characteristics of control and workers' groups

| Variable | Controls (N=40) | Workers (N=40) |
|---|---|---|
| **Sex** | Male | Male |
| **Age (year)** | 24-62, (35.88±8.65) | 23-62, (38.50±9.90) |
| **Height (centimetre)** | 163-195, (173.30±8.49) | 173-23, (173.23±7.22) |
| **Work experience (Year)** | - | 5-20 |
| **Taking medication** | 1 | 2 |
| **History of respiratory disease** | 0 | 2 |
| **History of drug use** | 0 | 0 |

There was no significant difference in demographic characteristics between the two groups

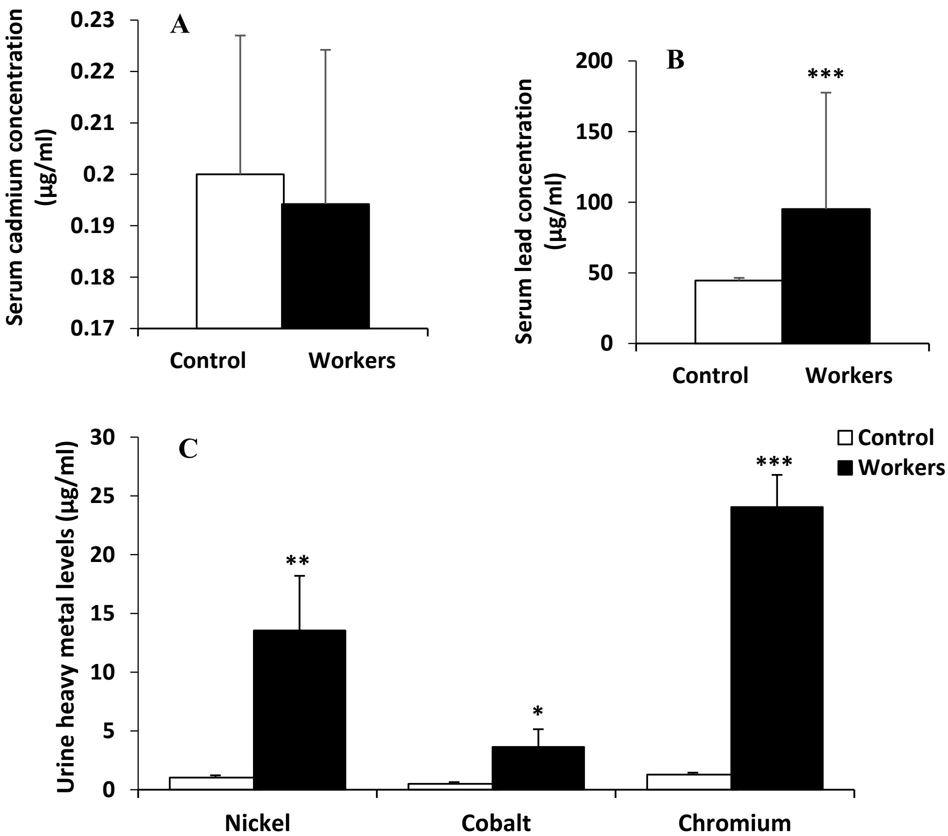


**Fig. 1.** The serum cadmium (A) and lead (B) concentrations and urine heavy metal level (C) in the control group (white columns) and workers of the cement factory (black columns). The values are presented as Mean ± SEM. *: $P < 0.05$, **: $P < 0.01$ and ***: $P < 0.001$ compared to control group. Statistical comparison was made using an unpaired t-test.

*Haematological and inflammatory markers*

The results presented in Table 4 indicate the differences in haematological parameters between two groups. The CBC test revealed that only MCHC ($p < 0.01$) and platelet counts

**Table 4.** Comparison of haematological results between study groups

| Variable | Normal range | Workers (N=19) | Controls (N=19) | P |
|---|---|---|---|---|
| **HGB (g/dL)** | 14–18 | 14.91±0.20 | 14.54±0.52 | 0.77 |
| **HCT (%)** | 41–50 | 43.61±0.47 | 44.92±0.49 | 0.07 |
| **MCV (fL)** | 80–100 | 86.83±0.88 | 87.22±0.88 | 0.76 |
| **MCH (pg)** | 27–31 | 29.67±0.29 | 30.11±0.26 | 0.57 |
| **MCHC (g/dL)** | 32–36 | 34.17±0.15 | 33.60±0.156 | 0.01 |
| **RDW %** | 11–15 | 12.56±0.14 | 12.77±0.154 | 0.34 |
| **PLT (count×$10^3$/mL)** | 150–450 | 249.05±13.13 | 217.26±4.50 | 0.02 |
| **MPV (fL)** | 7–12 | 9.47±0.19 | 9.10±0.15 | 0.15 |
| **PDW(fL)** | 8.1–25 | 15.87±0.09 | 15.46±0.19 | 0.07 |

The values are presented as Mean ± SEM. Statistical comparison was made using an unpaired t-test.
HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; PLT: platelets; MPV: mean platelet volume; PDW: platelet distribution width.

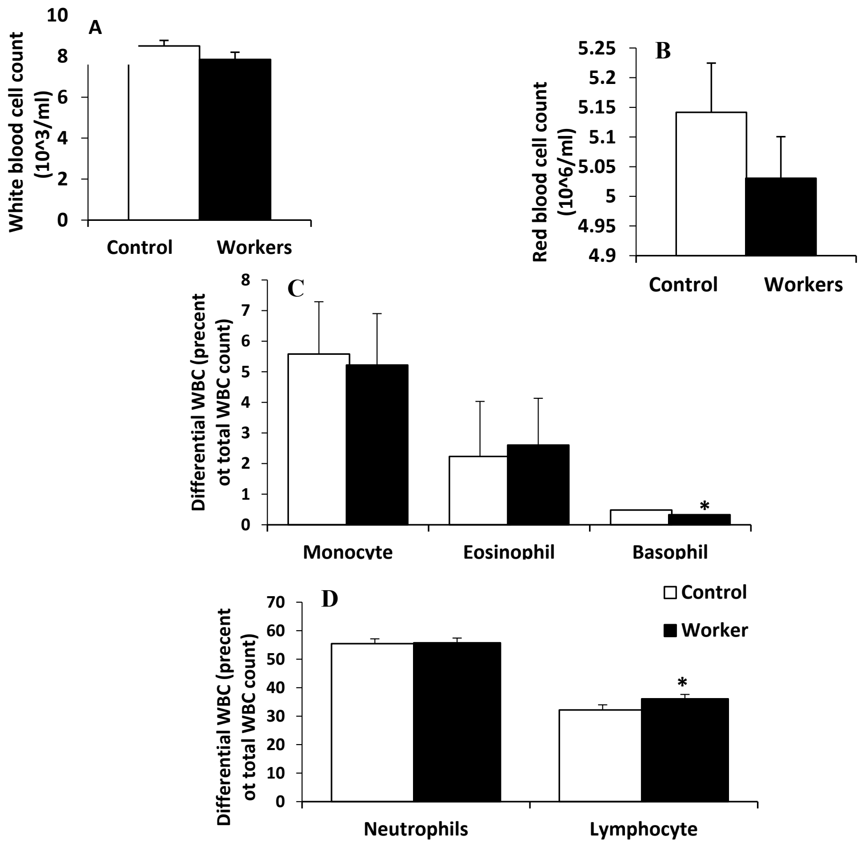


**Fig. 2.** Red blood cells (A), total white blood cell (WBC) count (B) and percentage of monocyte, eosinophil, basophil (C), neutrophils and lymphocyte (D) in cement factory workers (black columns) and the control groups (white columns). The values are presented as Mean ± SEM. *: $P < 0.05$ and **: $P < 0.01$ compared to control group. Statistical comparison was made using an unpaired t-test.

($p < 0.05$) were significantly higher in the workers compared to the control group. However, total WBC and RBC count, as well as other haematological parameters, were not significantly different between the two groups. However, the percent of lymphocytes was significantly higher, but basophils were lower in CF workers than in the control group ($p < 0.05$ for both cases), (Fig. 2 A-D).

The mean serum level of the inflammatory marker hs-CRP was significantly higher among the workers (1.21 ± 1.37 mg/dL) compared to the controls group (0.58 ± 0.44 mg/dL), ($p < 0.001$), (Fig. 3).

*Respiratory symptoms and PFT values*

None of the participants complained of sputum or dyspnoea, but other respiratory symptoms including wheezing at night ($p < 0.05$), coughing at night ($p < 0.05$), wheezing and coughing during exercise ($p < 0.001$), and wheezing during the days ($p < 0.001$) were significantly more prevalent in the workers' group compared to the control group (Fig. 4).

Pulmonary function tests indicated that FVC, FEV1, MMEF, MEF75, MEF50, MEF25 and PEF ($p < 0.05$ to $p < 0.01$) were significantly reduced in the CF workers compared to the control group (Fig. 5).

To the best of our knowledge, this is the first study evaluating the heavy metal concentration in serum and urine, inflammatory status, respiratory symptoms, and pulmonary function tests in cement-exposed workers. In the present study, the effect of exposure to cement dust in workers of a CF on respiratory symptoms, PFT, total and differential WBC count, and inflammatory mediators (hs-RCP), as well as urine and serum heavy metal levels were examined. The results showed a higher serum and urine heavy metal level, total and differential WBC counts, hs-CRP, and respiratory symptoms, but lower PFT values in the workers of the CF compared to the control group.

The cement industry is among the most important contaminating industries, and cement dust are one of the leading environmental concerns (AbuDhaise *et al*., 1997). Occupational exposure to multiple cement dust causes dermatitis, rhinitis, asthma, and chronic bronchitis (Carlsten *et al*., 2007). Several types of environmental pollution increase respiratory disease

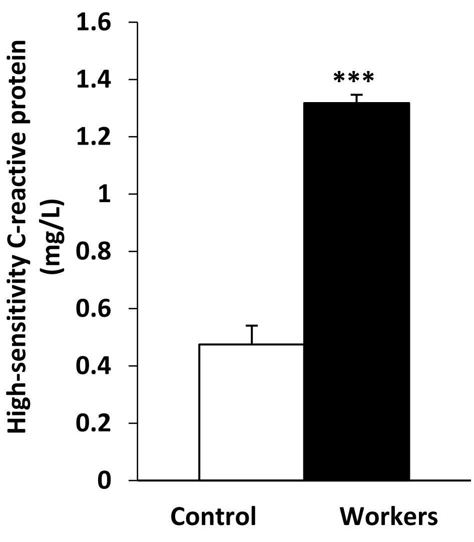


**Fig. 3.** The serum high-sensitivity C-reactive protein (hsCRP) level in the control group (white columns) and workers of the cement factory (black columns). The values are presented as Mean ± SD. ***: $P < 0.001$ compared to control group. Statistical comparison was made using an unpaired t-test.

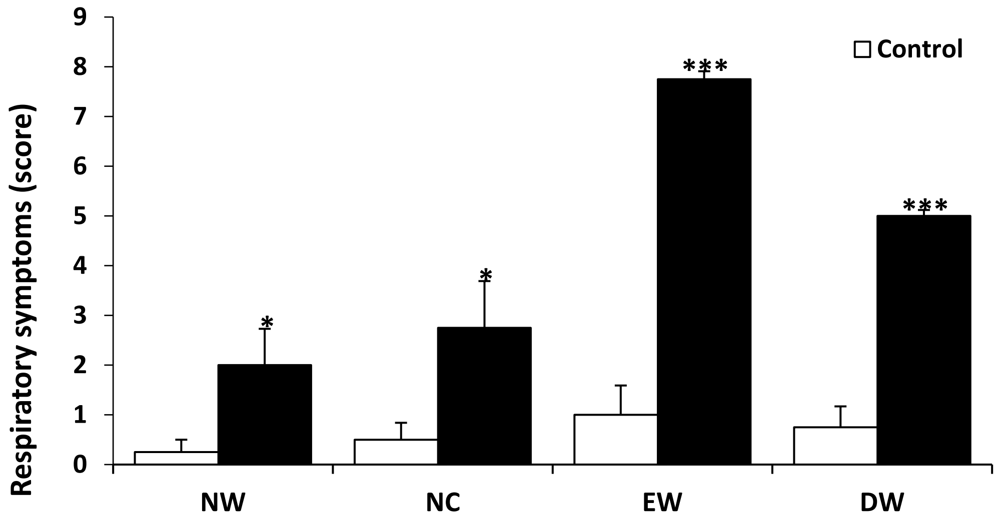


**Fig. 4.** Respiratory symptoms in the control group (white columns) and cement factory workers (black columns). The values are presented as Mean ± SEM. *: $P < 0.05$ and ***: $P < 0.001$ compared to control group. Statistical comparison was made using an unpaired t-test. NW: wheezing at night; NC: cough at night; ECW: wheezing and coughing during exercise; DW: wheezing during the days.

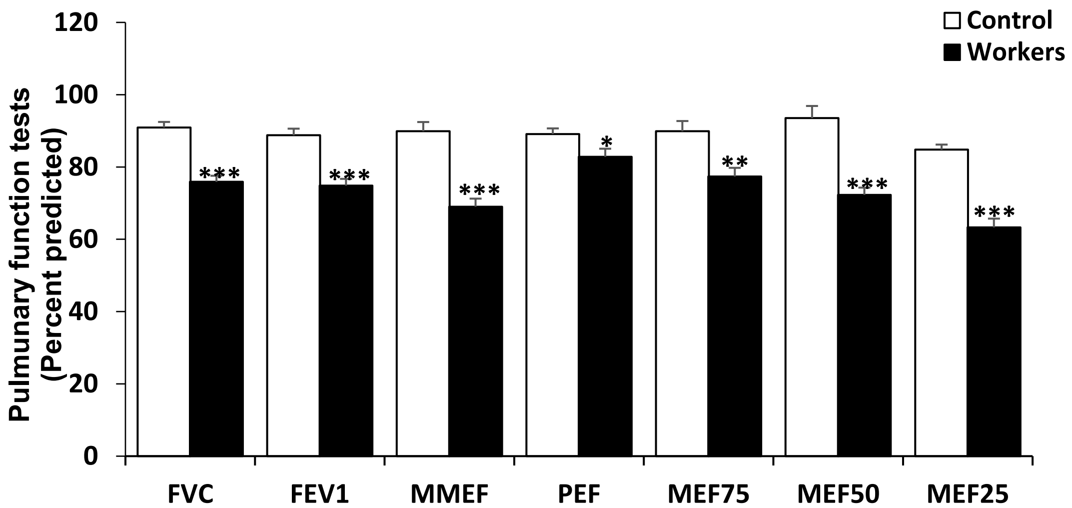


**Fig. 5.** Pulmonary function tests, including FVC, FEV1, MMEF, PEF, MEF75, MEF50, and MEF25 values in cement factory workers (black columns) and the control group (white columns). The values are presented as Mean ± SEM. *: $P < 0.05$, **: $P < 0.01$ and ***: $P < 0.001$ compared to control group. Statistical comparison was made using an unpaired t-test. FVC: Forced vital capacity, FEV1: Forced expiratory volume in one second, MMEF: Maximum mid expiratory flow, PEF: Peak expiratory flow, MEF25, 50,75: Forced expiratory flow at 25, 50 and 75% of the vital capacity, respectively.

risk, mainly COPD and asthma, which are considered a third respiratory disease that will cause death in 2020 (KAKOOEI *et al.*, 2012). Workers in the cement factory are significantly exposed to cement-containing crystalline dust (AminianAslani & SADEGHNIIAT, 2012). These workers are responsible for outspreading, blending, sanding, shedding, grinding hard concrete and producing cement dust (Mojiminiyi *et al.*, 2008). Chronic exposure to silica dust (quartz) increases the risk of respiratory disease, which is indicated by chest radiographic changes, decreased pulmonary function tests (PFT) and increased respiratory symptoms (M. Boskabady *et al*., 2014). Previous studies also have shown that exposure to cement dust can increase the risk of autoimmune diseases such as rheumatoid arthritis and lupus erythematosus (Flanagan *et al*., 2003; Society, 1997; Tjoe-Nij *et al*., 2003). These studies support the findings of the present study.

The results of a previous study also indicated the effect of cement dust exposure on liver function, serum levels of some elements, and heavy metals in the workers and people living near the cement factory (Parks *et al*., 1999). In the mentioned study, serum levels of arsenic, lead, manganese, copper, cadmium, selenium, and chromium in 50 cement factory workers and 60 residents near the factory workers were higher than 100 subjects who were not exposed to cement dust as a control group. Moreover, zinc and iron in workers were significantly lower than in the control group (Parks *et al*., 1999), which supports the results of the current study.

The results of a previous study indicated that serum level of some elements such as lead (Pb), copper (Cu), cadmium (Cd), chromium (Cr), and arsenic (As) were significantly higher in workers compared to controls subjects (Richard *et al*., 2016). The concentration of Pb and Cd in blood samples for cement factory workers in Sulaymaniyah Governorate, Iraq were higher than the control group (Hussein, 2023). The reason for the high Pb content in the environmental factory is the result of the high concentration of Pb in the cement. The results of mentioned studies indicated that contact with cement dust could result in increased heavy metals in the body.

It has been reported that all values of heavy element concentrations in blood samples from the worker and control groups are below the normal range (Naji & Hassoon, 2021).

In the present study, total and differential WBC counts and serum hs-CRP levels were measured in the control and the cement factory workers group to determine the effect of cement exposure on the inflammatory process. The results showed that only MCHC and platelet count were increased in the workers, but they were within the normal range. However, the serum level of hs-CRP and percent of lymphocytes were higher, but the percent of basophil was lower in 6cement workers than in the control group. Increased platelets and WBC count in the exposed group with cement dust were also shown previously (Mirzaee *et al*., 2008). However, the percentage of granulocytes and lymphocytes were not increased due to cement exposure in the mentioned study (Mirzaee *et al*., 2008). Previous studies also indicated that the number of neutrophils in the sputum specimen of workers during contact with cement was significantly higher than non-exposed individuals. Also, the percentage of neutrophils and lymphocytes in the blood of workers were significantly higher than the control group (Fell *et al*., 2010). However, the present study did not show any significant differences in the total number of WBC, neutrophils, monocytes, and eosinophils among the CF workers. Therefore, the finding of the present study and those from previous studies indicate that contact with cement dust caused inflammatory processes in the body.

The results of this study showed that all respiratory symptoms increased in the CF workers compared to the control group. Moreover, PFT values were reduced in the CF workers compared to the control group. In a cross-sectional case-control study, respiratory symptoms and PFT values, including PEF-FEF (25-75%), FVC and FEV1 have examined in the cement exposed workers. The results indicated no significant difference in the cough and wheezing symptoms between the control and workers groups. However, in the cement exposure individuals, all PFT values were significantly lower than the control group (Steenland & Brown, 1995).

Similarly, in 100 exposed and 120 non-exposed workers at the CF in Mashhad, Iran, showed that occupational exposure to cement dust could be a significant factor of respiratory system dysfunction (Rafeemanesh *et al*., 2015). Among the exposed group, respiratory symptoms such as cough (6% vs 0.8% of the non-exposed) and sputum (7% vs 0.8% of the non-exposed) were significantly more incident, while $FEF_{25–75}$% was considerably lower compared with non-exposed ones. Furthermore, $FEV_1$/FVC and $FEF_{25–75}$% negatively correlated with the duration of employment (Rafeemanesh *et al*., 2015).

Therefore, the results of this study and previous studies indicated that exposure to cement dust could significantly increase the level of heavy metals in the body. Increased concentrations of heavy metals in the body can lead to the activation of inflammatory processes. The inflammatory process may lead to lung disorders, which reduces PFT values and increases respiratory symptoms. In addition, exposure to cement dust can directly enter the lung through their inhalation and cause respiratory disorders.

The unique study outcome from this research is a comparison of respiratory symptoms, pulmonary function tests (PFT), total and differential white blood cells (WBC), serum level of high-sensitivity C-reactive protein (hs-CRP), and serum or urine heavy metal levels between 40 non-smoking male cement workers and 40 healthy volunteers.

## CONCLUSION

The results of this study indicated that exposure to cement dust in CF workers leads to increased serum and urine levels of heavy metals and systemic inflammation. Exposure to cement dust also can cause respiratory disorders indicated by increased respiratory symptoms and reduced PFT values. Increased serum and urine heavy metals may induce inflammatory processes leading to lung disorders. These results emphasise the continuous monitoring of workers' workplace safety and occupational status.

In the present study, the ordinary PFT values and most influential heavy metals were assessed. The measurement of DLco and other metals were the limitations of the current research and should be evaluated in further studies. Also, further studies in larger CF workers population with various duration in working, or people living near tCF, evaluating more heavy metal, and assessing the relationship among change in heavy metal, systemic inflammation, and change of respiratory symptoms and PFT values should be undertaken. In addition, the effects of different monitoring of workplace safety on the health status of CF workers should be studied.

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## CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

## LIFE SCIENCE REPORTING

The study was approved by the Ethical Committee of Mashhad University of Medical Sciences (Ethic code IR.MUMS.REC.1395.291).

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